

## Can and Should the Universities Survive?

UNIVERSITIES have very few friends these days. The students, for whom they are supposed to exist, are often the most fierce critics of the system. Governments and other sources of funds for higher education have often exchanged the enthusiasm for higher education fashionable only a decade ago for scepticism and parsimony. Employers are slipping into the habit of complaining that the kind of education which the universities provide is not that most likely to provide young men and women with the skills needed to keep the wheels of industry turning smoothly. And even among academics themselves, self-criticism is a growing preoccupation. How best to teach this or that? What, in any case, to teach? How best to bring students into the process of government? How should universities determine their relationships with the outside world? So common is this questioning that there is a danger that talk will become a substitute for change. With luck, of course, the outcome could be a valuable and constructive transformation of the pattern of higher education at least in Europe and North America, but most people will agree that care and patience will be needed if the adaptation of the university system to modern needs is to be accomplished without serious harm. So much is usually taken as common ground.

Against this background, there is great interest and not a little danger in a report published two weeks ago in the United States by a committee on higher education under the chairmanship of Mr Frank Newman, associate director of university relations at Stanford University. The report is likely to have an important influence on developments in higher education, not merely in the United States but wherever the rising demand for places in higher education has occasioned similar problems.

The Newman committee was established by Mr Robert Finch, President Nixon's First Secretary at the Department of Health, Education and Welfare. Two weeks ago, it was welcomed by Mr Elliot Richardson, Mr Finch's successor, as a refreshing statement of the problems to be tackled in higher education, and that certainly is true.

The report also breaks new ground by spelling out some of the questions which only occasionally find their way into words in genteel discussions. Is the pattern of the university curriculum in the United States, derived as it is from the academic patterns of the older universities, properly suited to the needs of modern students? Should universities (in North America) recruit simply from the ranks of elderly teenagers? Must they discriminate against women? And blacks? Must growth mean that universities become gigantic bureaucracies? Is it right that conventional academic awards should be the sole tests of academic success? Is the tenure of academic appointments an indispensable part of academic freedom? In circumstances in which scepticism may often be constructive, all this is sensible. The danger is that the committee's statement of the problem will become a quarry of brickbats to throw against the universities, and that by creating a sense that change is merely a matter of commonsense, without describing detailed mechanisms by which desired

change might be accomplished, the report will increase the impatience of those who are looking for signs of change.

The committee's argument starts from the simple observation that there is no necessary equivalence between attendance at a university or similar institution and the process of gaining an education. That, of course, is a simple truism, although Dr Newman and his committee have been able to make their readers shudder splendidly with frightening statistics of the drop-out rates from universities in the United States. The way things are going, less than half of the million people each year who enter higher education in the United States will stay the course for two years, and only a third will stay for the regulation four years. But drop-outs are not simply flunk-outs—they are customers dissatisfied with the academic diet with which they are provided. But on the other side of this coin, according to the committee, is the danger that students will be locked in the system so that they are carried along, against their better judgment, from one degree course to another until they emerge from graduate school robbed of their native capacity to live successfully in the real world, and even badly educated for it as well. So "to what extent should the nation commit itself to increasing the number of college and university places and the availability of student aid if, by so doing, it provides access to a refuge rather than to an education?" As a rhetorical question, this is worth asking, but there are many who will interpret it not as the statement of a problem but as a statement of fact.

The committee is also fierce on what it calls the homogenization of the university system in the United States, and there are many academics who will benefit from the reminder that the once distinctive characteristics of institutions such as the land-grant colleges have been submerged only in the past few years by the traditions of the older universities. The Newman committee also wrings its hands about the decline of colleges and universities originally created to serve the needs of special interests—religious sects, ethnic groups, those wanting to be lawyers or teachers and the like. This is good tear-jerking stuff, yet there are many readers who will ask whether the institutions of the good old days would ever have been able to accommodate the virtually universal higher education now within sight in the United States. Sheer growth has been one powerful agent of this past change, and if the committee wishes to assert that matters could have been differently organized, it must provide some kind of proof. By the same test, it must make a proper evaluation of the benefits which there have been in past decades in a system which has enabled students from higher schools of all kinds, many of them bad high schools, to try out their heads in an academic world. In other words, there is a sporting chance that the freedom with which students have been able to move from one institution to another, and even the traditionally high drop-out rate at the less selective universities, have been essential to the upward and lateral social mobility of the United States since the twenties. This is a point which the committee entirely overlooks.



But are circumstances now different? This is an entirely proper question to ask. One of the committee's strongest arguments is for greater diversity within the system. It would like to see more opportunities for part-timers to be educated (which is sensible enough). It would encourage diversity in age of entry to the university system (which is also sensible). And it makes a strong plea for the creation of "new educational enterprises"—colleges providing instruction in special fields or with curricula designed so as to make more fruitful the relationship between the university and the community. All this, in theory, is sensible enough, and the committee acknowledges that there have been in the past few years some valuable experiments in the United States. Yet it remains a sobering truth that experiments like these, however well intentioned, are not always successful. One of the lessons of the past few years is that innovation in educational institutions is bound to be a slow and even painful process. One of the obvious pitfalls is that attempts to match the objectives of higher education with desirable social goals may frequently be frustrated by ignorance and even idealism. Even in the attempts now being made to provide more or less traditional curricula by conventional methods, as in television and broadcast universities such as the British Open University, it is by no means certain that the wish will always be the father of the thought. In this sense, too, the Newman committee has taken too little account of the difficulties of change and of the penalties of failure in its impatience to light a fire under traditional university institutions. This is a great misfortune, if only because it may encourage wild innovation while traditional academics scuttle to their tents and sulk.

The essence of the Newman problem, after all, is to define the function of higher education in the modern world. There is more to be said than that the universities and other institutions are means of keeping young people off the streets until they have a chance (and the inclination) to contribute to the national economy. The same problem overshadows the development of higher education throughout Western Europe, not to mention Japan and Latin America. One of the first things to acknowledge is the vocational function of even the most ancient and learned of universities. The contribution of the universities towards vocational training in the modern sense is that they have been able to provide a framework of scholarship within which young people can acquire not merely conventional skills but inventiveness as well. In its polemic against the present system, the Newman committee is far too neglectful of this powerful truth.

It is also shockingly carried away by the fashionable belief that the PhD degree is an impediment to useful work.

With these reservations, there is much to be said for the thread running through the committee's report that the time has come to redirect the system of higher education in such a way that the vocational needs of students are more openly acknowledged. If universities were as confident of their achievements as they should be, this declaration would not seem a threat to scholarship. The history of the past few decades is as powerful a reminder as could be sought of how scholarship can contribute to practical works. Curricular changes are, however, necessary, especially in the last two years or so of a university course, and it is fair to say that the experiments so far have been tentative, to say the best of them.

With such a goal, diversity is of course essential. It is hard enough for each university—in Britain or the United States—to provide itself with a department of biochemistry or of classics. To provide means of helping people to train as teachers, or to operate computer systems, is beyond the scope of all but the largest, and there are no means of knowing whether Dr Clerk Kerr's "multiversity" is more or less desirable than a collection of smaller institutions with differing interests and ways of doing business. Most probably, the balance of advantage will usually lie with the collection of smaller institutions, if only because other arrangements will be less flexible, but this implies that students will frequently wish to transfer from one institution to another. There are also problems of making sure that imitative competition among institutions, together with the movement of proselytizing teachers from one kind of institution to another, does not iron out differences once established. These, however, are not immediate obstacles. The more urgent need is somehow to arrange that academic institutions are organized into patterns within which students can move, if necessary, from one place to another that seems quite different, and within which academics will also be liberated from the sense of being bound to one predetermined style of teaching and research. In Britain, for example, what this implies is that there should be federal associations of universities, polytechnics and teacher training colleges and not the three separate systems which now coexist. In the United States, the more urgent need is to find some way of allowing universities and other such institutions to cultivate their own distinctive character. For all its faults of polemic and of neglect, the Newman committee has done well to hammer home this point.

## European Technology in Dissarray

WHAT has happened to the brave prospect that Europe would be united by technological necessity even if, for reasons beyond most people's control, it should prove impossible to bring about some kind of political understanding between countries whose interests are necessarily diverse? After all, it is less than a decade since the then British Government sought to make its path into Europe smoother by arranging to build the Concorde aircraft in collaboration with France, since the rocket called Blue-streak was made the altar for celebrating—or is it sacrificing?—the brave dream of a European programme of

space development. More recently, there has been the attempt within the EEC to spell out ways in which there could be collaboration among several nations on projects which are usually too big for nations to tackle on their own. Not so long ago, M Aigrain, chairman of most of the working groups concerned, was hoping to arrange collaborative projects within the EEC in fields such as telecommunications, the construction of large computers and even the investigation of pollution and the methods by means of which pollution should be controlled. Now (see page 200), less ambitious schemes are the order of



the day. What has gone wrong? Has the dream of technical collaboration been defeated by political necessity? Or by inner logic or the lack thereof? Or is it merely that people in Europe have come to take a much more realistic view than even a few years ago of what can and cannot be accomplished?

The first thing to be said is that there was a great sense of unreality about the schemes hanging in the air three years ago, when technical collaboration on the grand scale seemed not merely a means by which the whole could be stronger than the sum of its parts, but when there was also a great preoccupation with what was then known as the technology gap, the apparent disparity in technical skill between Europe and the United States. The chief difficulty was the way in which the imagination of European technocrats appeared to leap ahead to the point at which Europe, too, will be as powerful an industrial enterprise as the United States itself. The tendency was to say that if the United States was industrially powerful enough to sustain and to profit from a flourishing computer industry, surely it would be possible for Europe to emulate her competitor by finding ways of making computers to an advanced design. By the same logic, people were impelled to think of schemes for being preoccupied with pollution. If the United States is rich enough to be able to afford such luxuries, so the argument seemed to go, then will it not profit Europe as a whole to make some show of following suit? The trouble, in this particular case, is that the particular proposals put forward by the EEC for collaboration on pollution were devised not merely so as to carry out useful scientific work, but to find some use for laboratories no longer needed within Euratom (which should itself have been a warning of the dangers of ill-constructed schemes for collaboration). In all kinds of circumstances, in other words, there was a great temptation to put the cart before the horse.

But where should people look for opportunities in collaboration? This is the question not yet resolved in any of the schemes so far suggested. The easiest formula seems always to entail some rigid partition of labour between one company or organization in one country and some partner elsewhere. This is the way in which the Concorde is being developed, for example. Both partners profess their belief in the principle that technical development is not an end in itself but merely a means to an end (which in this connexion is usually prosperity), yet when it comes to the development laboratory, each country wishes to do some of the work itself for the experience it may acquire. The result, of course, is that the partners in these enterprises spend more collectively than they would have done if they had been working separately. There are even qualitative snags in attempting joint projects for development—inefficiencies, misunderstandings and even open rows. Yet the durable benefit of whatever collaboration is achieved is that the partners are able to distribute the development cost over a market which is at least a little larger than if both of them had insisted on going their own way independently when, as with the development of nuclear power in Europe, each of them would have rather died (or in practice bought American) than allow itself to be seen buying from its closest rivals. But does it not follow from this that the best route to European collaboration in technology is not to arrange for artificial projects in which carefully chosen teams of partners, governments and companies, all mixed together, embark on some project regulated by

treaty, but to ensure that European organizations with something new to sell have access to the European market for sales and to the European labour market as well?

The moral, in other words, is that to bother too much at this stage about the forms and ceremonies of collaboration in technical development may be mistaken. Why seek to accomplish artificially what will presumably follow naturally once the EEC is a going concern in the fullest sense? At present, after all, the full rigours of the Treaty of Rome are not applicable even within the EEC. Labour still does not move freely, and the movement of capital is still restricted. More serious, nations are still anxious to buy what they make themselves whenever this is possible, and no doubt the temptation will persist at least until there are ways of making sure that countries within the EEC, deprived of their jobs from time to time by the shifting pattern of changing industry, are able to lay off some of the damage done, presumably by means of continental social security programmes. All this implies that full technical collaboration in Europe is not so much a matter of deliberate arrangement as of political and social engineering.

## 100 Years Ago



### FIRST REPORT OF THE ROYAL COMMISSION ON SCIENTIFIC INSTRUCTION AND THE ADVANCEMENT OF SCIENCE.

TO THE QUEEN'S MOST EXCELLENT MAJESTY.

*May it please your Majesty—*

WE, the Commissioners appointed by Your Majesty to make Inquiry with regard to Scientific Instruction and the Advancement of Science, humbly beg leave to present to Your Majesty the following First Report:—

1. We have heard the evidence of witnesses in reference to the following subjects, forming part of our inquiry, viz., the Royal School of Mines, the Geological Survey of Great Britain and Ireland, the Mining Record Office, and the Museum of Practical Geology, at present located in Jermyn Street; and also concerning the Royal College of Chemistry, at present lodged in a building in Oxford Street; which institutions are under one head, entitled Director-General of the Geological Survey of Great Britain and Ireland and Director of the Royal School of Mines.

2. There is no necessary connection between the direction of the Geological Survey of Great Britain and Ireland and the government of the Royal School of Mines.

3. The Royal School of Mines and the Royal College of Chemistry, which practically constitute one School of Pure and Applied Science, are not organised in such a manner as to enable them to perform efficiently the work for which they were originally, or are, at present, intended. We base this conclusion upon three grounds, (a) The absence of a chair of Mathematics, (b) The absence of Physical or Biological Laboratories in which students can receive practical instruction, (c) the insufficiency of accommodation in the Royal College of Chemistry.

*From Nature, 3, 421, March 30, 1871.*

## OLD WORLD

### COMPUTER POLICY

## Whistling in the Dark

A BRIGHTER picture of the future was painted by International Computers Limited before subcommittee A of the Select Committee on Science and Technology last week than might have been anticipated from earlier sessions, when members of the subcommittee expressed doubts about ICL's ability to raise sufficient research expenditure to match what is being spent elsewhere, particularly by IBM. Sir John Wall, chairman of ICL, and Mr Arthur Humphreys, managing director, professed themselves happy with the Government's new policy for the purchase of computers as explained to the subcommittee by Sir John Eden of the Department of Trade and Industry (*Nature*, 230, 4; 1971), and they were not disturbed by the approaching end of Government support to ICL under the merger agreement. ICL's research expenditure is met from each year's output, Sir John Wall said, and so long as ICL continues to receive orders, there will be finance for research and development work.

But to a question from Mr Ian Lloyd, Mr Humphreys estimated that a 15 per cent increase in ICL's turnover per annum is needed to generate the increase in research expenditure that the ICL management would like to see. To show that there is nothing to worry about, the ICL representatives came armed with figures showing the progress that has been made since the merger that formed ICL in 1968. In the year ending on September 30, 1970, turnover rose by 13 per cent to £130.8 million, and the profit after tax by 33 per cent to £4.5 million, which apparently is in tune with the predictions made three years ago, and the System 4 range of computers inherited from English Electric Computers is no longer making a loss. ICL is also proud of making a positive contribution to the trade balance in computers of £20 million last year, compared with a total trade deficit in computers of £70 million.

Nevertheless, the ending of investment grants in October last year has not helped ICL. Coupled with the latest rounds of wage increases, the effect has been that companies are deferring their expenditure on computers. The ICL representatives estimate that the industrial market has gone down by about 25 per cent as a consequence, although ICL is confident that eventually management will realize that the only way to pay the higher wages that are being demanded

is to be more efficient and this will stimulate the use of computers. Sales of machines to local authorities and to financial concerns are not being depressed to the same extent because, according to ICL, these organizations appreciate the value of computers in their businesses.

ICL is also concerned about interference to their trade in Eastern Europe, caused by delays in obtaining assurances that the material to be exported does not come under the embargo against the export of strategic materials. Sir John Wall said that it can take a year to obtain a licence, although ICL is hoping that the orders for Serpukhov will go ahead. But he admitted to a suspicion that the authorities in Washington are dragging their feet.

### AIR TRAFFIC

## Growth Rate Forecast

WHILE public attention has focused on the battle over the siting of London's "third" airport, little notice has been taken of the continuing growth of the five airports in the London area. To be sure, a small but vociferous group has claimed that the existing airports should be sufficient for London's immediate needs, if the development of short and vertical take off and landing aircraft is considered. But the claim has lacked the vital support of official statistics concerning the prospects for air traffic in the London area during the critical next decade. Now, the British Airports Authority has produced forecasts of the growth of traffic through Heathrow, Gatwick, Stansted, Luton and Southend airports during the period 1970-85.

Overall, the forecasts predict a growth in passenger traffic from 22 million to between 80 and 130 million passengers per year in the fifteen years. Similar forecasts of air freight requirements suggest that the present yearly cargo tonnage of 400,000 tons will grow to more than 2.5 million tons, and possibly to as much as 6 million tons, while the demand for air transport will double the present 350,000 flights per year. These forecasts show higher growth rates than those used in the calculations of the Roskill Commission, which investigated the siting of London's third airport, primarily because the authority has anticipated a greater increase in the movement of all-cargo aircraft. Also, charter flights should continue to take a growing share in the movement of passengers, with roughly 6 million passengers a year travelling by charter flights to North America alone in the early 1980s.

But what bearing are these figures likely to have on the eventual decision of the Government on the siting of the third London airport? One interpretation could result in a panic to get a

new airport built more quickly than the Roskill Commission has recommended, but surely a wiser view would be that the need for a national airports plan is becoming increasingly clear. The problem should not be tackled piecemeal.

### EUROPE

## PREST Depressed

*Paris, March*

POLITICAL problems continue to dog the uncertain footsteps of the European Community's Scientific and Technical Research Policy group (PREST). It now looks as if few of the ambitious proposals for European collaboration in research which were put forward two years ago will be put into effect. Of the original forty-seven projects, only twenty remain extant, and the anticipated level of investment now stands at only 30 million dollars, a far cry from the 150-500 millions originally mooted. Of the principal projects, which included the construction of a giant computer of revolutionary design, only the European Centre for Meteorological Computing has survived the axe.

PREST, known colloquially and successively as the "Marechal" and "Aigrain" group after the names of its two French presidents, outlined the list of projects for collaboration in a report published in March 1969—a considerable diplomatic achievement when three months earlier only the efforts of M Michel Debré had eased the somewhat tense situation between the ministers of the six member countries. But there has been little advance since then.

What has gone wrong? The chief obstacle is the awkward question of whether the European Community is qualified to develop its own research activities except in the field of nuclear technology. And then there is the very reasonable concern about the collaboration of non-member countries in certain projects. Reaching agreement between six countries is difficult. When fifteen or more are involved, there seems to be no end to the arguments.

The situation seems unlikely to improve much by July 1971, when the Council of Research Ministers will meet for the first time. The ministers of education are also to meet to discuss the exchange of scientists within the community, but here it is practical problems, such as the standardization of professional training, pay, and retirement policies, rather than political obstacles which will be the hardest to overcome.

### POLLUTION

## Councils Co-operate

FAR more cooperation between the British research councils and increased



support for fundamental research are essential if the problems of pollution are to be tackled efficiently. This is the message of a report published last week by an inter-council working party, which defines concisely the responsibilities of each council and proposes the establishment of a liaison group, to collate the research programmes of the councils and refocus their attention on neglected aspects of pollution.

Although pollution research comes under the auspices of all five councils, the Agricultural Research Council, the Medical Research Council and the Natural Environment Research Council inevitably support the bulk of the direct research (see Table). The report emphasizes, however, that the life blood of the research as a whole is the background, or indirect, research supported principally by the Science Research Council and the Social Science Research Council. The councils spend at least £1 million a year on research directly related to pollution, and a further £2 million on indirect but relevant research.

There are, of course, strong links already between the research councils and Government departments. Indeed, the direct research financed by the councils, being chiefly concerned with the long term effects of pollution, complements and enlarges the shorter term investigations of the Government laboratories.

The report is more concerned, however, with the areas of overlap between the councils and makes several proposals for more concerted action. Fundamental toxicology, public health and industrial pollution are three topics to which the SRC, for example, could contribute more by stimulating fundamental research. The report also points to a shortage of information about the effects of air pollution and to the lack of a focal point for research of this kind. This is a case, it says, for co-operation between the ARC, the NERC and the Government departments concerned with agriculture and forestry. Both the MRC and the NERC support research into the effects of heavy metals on the ecosystem, but links with the Government Chemist and other Govern-

ment laboratories should be nourished.

In an effort to promote concerted action by the research councils, a member of the headquarters staff of each council has been given special responsibility for pollution research. Each will be kept abreast of the relevant activities of his council and will be a member of the Inter-Council Liaison Group on Pollution.

## ENERGY

### Comeback for Coal

AFTER a period of plenty in world energy resources, Western Europe is entering a decade in which the continuing growth of demand for fuel, combined with the hard line now being taken by the authorities in many oil producing countries, will produce an increase in the price of fuel great enough to make the full utilization of coal reserves desirable and economically worthwhile. After the Suez crises, two effects helped to ensure the dominance of oil and natural gas as the prime sources of energy. At the same time as the United States reduced its importation of oil, a wave of exploration revealed new fields in the Middle East, Africa, Alaska, and even Europe. The plentiful supply of reasonably cheap oil led to a rundown of the coal industry in Britain and Western Europe which made economic sense in the short term, but which may have unpleasant consequences unless the trend away from coal can be reversed.

Today, the world consumption of fuel is 7,000 million tons of coal equivalent (mtce); by 1980 the demand may be as great as 12,000 mtce, and nuclear power is unlikely even to cope with the growth in demand, let alone usurp the role already played by conventional power stations (see *Nature*, **230**, 138; 1971). Yet during the period 1960 to 1970, the United Kingdom and the European Economic Community between them cut back annual production of coal by some 160 million tons. Now 60 per cent of the energy requirements of the EEC are met by imports, and even in the UK only just over half (56 per cent) of all fuel requirements is met from native resources.

The prospects for the future, presented in *Energy Resources for Western Europe* (Association for Coal in Europe, 1971; 75p), seem to depend heavily on how quickly the coal mining industry can be revived. Ironically, this most primitive form of fuel will, it seems, take the place which had once appeared to be reserved for nuclear power for at least ten years. After this period, nuclear power must become a dominant contributor if the estimates of a world demand for 25,000 mtce by the end of the century are to be met. So it

seems that the champions of the coal industry were right to raise their voices against the change to other fuels.

## Parliament in Britain

### Student Grants

ALTHOUGH maintenance grants to university students in residence at London, Oxford and Cambridge rose by more than 6 per cent between June and September 1970, by January 1971 the real value of the increase in terms of purchasing power had fallen to less than 2.5 per cent, said Mr William van Straubenzee in reply to a question from Mr Golding. The comparable figures for students at other universities were 5.6 per cent and less than 1.7 per cent. For resident students at colleges of education, the increase in the value of their grants has been almost completely eroded away by the decline in purchasing power. (Written answers, March 16.)

### Cancer Research

A SCHEME of scientific interchange through which a member of the US National Institutes of Health will be seconded for some time to the headquarters of the Medical Research Council while a senior member of the MRC staff will visit the International Agency for Research in Cancer, Lyons, and various other centres, including NIH, will be the first tangible sign of the promised British co-operation in the international attempt to find a cure for cancer, said the Prime Minister. The cost will be met from MRC funds. Mrs Thatcher, Secretary of State for Education and Science, said that the MRC's direct expenditure on cancer research is currently £2,166 millions yearly. (Written answers, March 16.)

### Exhaust Fumes

AFTER campaigning so imaginatively to prevent increases in the road fund tax, Sir Gerald Nabarro seems now to be in the vanguard of those who seek to protect the purity of the air. He asked what benefit to the health of the population, and in consequence what reduction in the nation's health bill, could be expected over ten years if £2,500 million or £150 per car were spent over the same period on devices to remove pollutants from the exhausts of motors. Mr Alison, Under-Secretary at the Department of Health and Social Security, was less enthusiastic. He said that although the possibility of undetected hazards could not be excluded, there was little to suggest any danger to health from street concentrations of motor fumes. It is impossible to say what improvement in health might result from removing pollutants from the fumes. (Written answers, March 16.)

Estimated Direct Expenditure on Major Types of Pollutants

| TOPIC                          | ARC | MRC | NERC | SRC | SSRC | Total |
|--------------------------------|-----|-----|------|-----|------|-------|
| General investigations         | —   | —   | —    | 17  | 21   | 38    |
| Organic wastes                 | 48  | —   | 69   | 9   | —    | 126   |
| Bacterial/viruses/spores       | 25  | 3   | 9    | 9   | —    | 46    |
| Pesticides                     | 110 | 3   | 60   | —   | —    | 173   |
| Oil                            | —   | —   | 27   | 4   | —    | 31    |
| Particulates/hydrocarbons/dust | —   | 33  | 4    | 1   | —    | 38    |
| Noise                          | 13  | 4   | —    | 28  | —    | 32    |
| Radioactivity                  | —   | 272 | —    | —   | —    | 285   |
| Heavy metals/chemicals         | 2   | 26  | 32   | —   | —    | 60    |
| Unclassified                   | —   | 168 | 20   | 6   | —    | 194   |
| Total                          | 198 | 509 | 221  | 74  | 21   | 1,023 |

Figures are in thousands of pounds per annum, and refer to 1970-71.

## NEW WORLD

### RESEARCH SPENDING

## More Numbers Games

THE National Science Foundation seems to be the latest recruit to the ranks of those who think that it can only be a matter of time before expenditure on research and development in the United States begins to grow again as in the fifties and early sixties. Only this or sheer silliness can explain some forward projections of expenditure which appear in a report now circulated by the foundation under the title "National Patterns of R & D Resources" (US Government Printing Office, 50 cents). What the foundation has done is to work out the amounts that will be spent on research and development by public and private sources during the seventies, with the assumption that research and development will take a constant proportion of the gross national product. The result is the cheerful prediction that by 1980, research expenditure measured in 1969 dollars will be something between \$33,700 million and \$44,500 million, compared with \$27,800 million in 1971. The snag, of course, is that much of the substance of the same report is a detailed demonstration that the old ratios are quickly being eroded.

Research and development expenditure is now growing less quickly than the GNP, stagnant though that may be. In the past five years, for example, the GNP has managed to grow by 6.9 per cent a year, but total expenditure on research and development by only 4.6 per cent a year on average. The result is that the proportion of the GNP spent on research and development has fallen steadily from its peak of more than 3 per cent in 1964, and is estimated to be in the current year just under 2.7 per cent. To the extent that the magic figure of 3 per cent was in the early sixties the yardstick by which nations other than the United States were inclined to estimate their own success, the abandonment of this norm may easily be a public benefit.

Much of the interest of the report stems from its estimates of research and development expenditure by industry, the outcome of regular surveys by means of questionnaires. It now seems that industry has done a good deal to make up for the increasing parsimony of the Federal Government in the past few years. Thus while the total spent

by the Federal Government on research and development has been declining by about 3 per cent a year since 1966, funds for research and development from non-federal sources have been growing by 5.2 per cent a year (both figures measured in 1969 dollars). The report says that in the non-federal sector, industry accounts for 71 per cent of research expenditure and that some 60 per cent of this money comes out of company funds. Moreover, the report suggests that this pattern is likely to persist throughout the seventies. Total expenditure by United States industry on research and development has now increased to more than 1 per cent of the GNP, and was in 1970 as much as a third of what was spent by manufacturing industry on new plant and equipment and rather more than a half of advertising expenditure by the same organizations. Predictably enough, most of the public money that finds its way into industrial research and development is spent on aircraft and missiles (53 per cent), but industry's contribution to its own research is much more evenly spread (see Table).

**Table 1** Uses of Federal and Company Funds in Industrial Research (Percentages)

|                       | Federal funds | Company funds |
|-----------------------|---------------|---------------|
| Aircraft and missiles | 53            | 13            |
| Electrical equipment  | 27            | 20            |
| Machinery             | 4             | 14            |
| Chemicals             | 2             | 16            |
| Motor vehicles        | 5             | 12            |
| Others                | 9             | 25            |
| Total                 | 100           | 100           |

Inevitably, the greater part played by industry in the financing of research implies that a smaller proportion of the funds available are spent on basic research. Industrial expenditure on basic research has in fact increased by only 3 per cent since 1966, while the total volume of industrial research and development has increased by 25 per cent. Paradoxically, however, there has also been a decrease over the years in the real cost of keeping a man (or woman) occupied in research and development. For the United States as a whole, according to the report, the real annual cost (measured in 1958 dollars) of research and development was \$32,700 in 1961, \$37,000 in 1965 and \$36,800 in 1969. Although these comparisons are innocent of ways of measuring the tangible value of one

man's work, so that the cheaper research may have been genuinely less productive, they raise awkward questions about the assumption that the increasing sophistication of scientific research makes necessary a steadily rising cost per man.

The National Science Foundation has also made public the results of a survey which shows how the stringency of the past few years has affected the balance of scientific work in United States universities ("Impact of Changes in Federal Science Funding Patterns on Academic Institutions", US Government Printing Office, 75 cents). The document consists of a comparison of the replies to two questionnaires completed in 1969 and 1970 respectively by a sample of the universities granting PhD degrees and by the chairmen of departments in fields such as the physical sciences, psychology and even economics. An important feature of the survey is that it deals not merely with research and graduate education, but with the entire operation of university science departments.

The large private universities, once again screwing up courage to sell off more of their endowment, will not be surprised to learn that although there was a 2.5 per cent increase of federal expenditure in university science departments between 1969 and 1970, and public institutions such as the state colleges received an extra 5 per cent, the federal contribution to the private universities actually declined by 1.5 per cent. Roughly half of the universities covered by the survey (86 out of 104 invited) were, however, able to compensate for the shortage of federal money by raiding other sources, chiefly subventions from state governments and increases of tuition fees. But the private universities were inevitably more dependent on tuition increases and thus less able fully to compensate for the decline of federal expenditure, reckoned to contribute about 40 per cent to the total costs of university research and education in science at the universities with graduate schools.

Most science departments seem to have been able to scrape together some extra funds between 1969 and 1970, and 11.7 per cent of the 651 science depart-

Our Washington Correspondent is on vacation.



ments covered by the survey even increased their spending by 25 per cent or more from one year to the next. Among the 29 per cent of university science departments with less to spend, however, there are significant variations. Physics departments fared badly, with 39.4 per cent of departments having to draw in their belts between 1969 and 1970. Biochemistry departments come next (38.1 per cent), followed by microbiology (34.1 per cent) and the departments loosely called biological sciences (32.8 per cent).

The precise meaning of these figures is not immediately apparent, chiefly because the effects of recent privations are mixed up with the unavoidable ups and downs of departmental fortunes. It must, however, be significant that mathematics departments were not seriously affected, with only 16.2 per cent having to draw in their horns, that economics departments did better still (with only 14.3 per cent having less to spend) and that psychology departments seem still to be booming away, with 74.2 per cent having more to spend and 19.4 per cent even having budgets increased by more than 25 per cent. In the changing pattern of the source of money from federal to non-federal, there is no decisive evidence in the statistics that state governments are rallying principally to the support of the softer sciences, but it may be a sign of things to come that of the university departments with less federal money between three-quarters of the economics departments concerned and two-thirds of the mathematics departments were able to make good the damage from other sources, but that physiology and physics, for example, were much less able to make good the damage.

#### GENEVA PROTOCOL

### Bundy against Herbicides

MR MCGEORGE BUNDY, president of the Ford Foundation and previously national security adviser to Presidents Eisenhower and Kennedy, made a powerful intervention last week in the debate about the ratification of the Geneva Protocol. Mr Bundy told Mr Fulbright's Foreign Relations Committee in the Senate that it would be wrong for the United States to attach to the protocol, now coming up for ratification nearly half a century late, a statement reserving the right to use herbicides and tear gas in circumstances such as those in which these agents have been deployed in Vietnam. Tactically, Mr Bundy suggested, the Foreign Relations Committee of the Senate should try to postpone action on the protocol until the Administration has

had a chance to reconsider the wisdom of a reservation. He and the committee have it clearly in mind that the Administration has embarked in the past two weeks on a fresh study of the military usefulness of herbicides and tear gases, and that there is on this occasion every chance that the result will be a revision of official policy. The Foreign Relations Committee will have heard this week from Assistant Secretary Nutter at the Department of Defense, who cancelled an earlier appearance before the committee in favour of a luncheon appointment, and from Professor M. Messelson of Harvard University, who is entitled to a large part of the credit for the substantial liberalization of the United States position on chemical and biological weapons. Evidently there will be very little difficulty in dragging out deliberations within the committee until the Administration has had a fresh chance to consider where it stands.

Mr Bundy's declaration was forthright as well as guileful. He said last week that he knew of "no military commander who would claim that in the wider perspective of the course of the war as a whole, their value has been at all critical". He said that the original decision to use herbicides in Vietnam had been predicated on the need to clear jungle trails, and that "as time passed, increasingly warlike uses were found for them". The problem, he said, was that what has happened in Vietnam with herbicides and tear gas is "what tends to happen quite remorselessly in war—unless there are very sharp and clear defining lines against the use of a given weapon, it tends to be used".

#### SUPERSONIC TRANSPORT

### No Peace for the SST

THE great supersonic transport row continues unabated, and although the chances are that the Administration will somehow be able to wring from Congress money with which to keep the Boeing development alive, the past few days have given those active on behalf of the project more serious cause for alarm than at any time in the past two years.

Matters came to a head on March 18 when the House of Representatives surprised the Administration and even itself by voting down a resolution that would keep the SST project in being for the last quarter of the financial year that ends this June. By now, it should be known whether the Senate will on Wednesday have followed the House of Representatives, and its own precedent in 1970, by voting against the programme of development. Those

with their hearts in the programme were earlier this week almost bewildered by the prospect of a sudden halt in this considerable enterprise. "I just can't believe that Congress will say no," said one of them.

Yet the most sombre warning for those wishing to keep the supersonic transport project alive must be the way the argument against the project has come to rely increasingly on the economic case against the SST, and to be less and less dependent on the argument that supersonic transport aircraft will cause deleterious changes in the upper atmosphere once they are in service. However the tussle about funds for continued development is resolved, the growing anxiety about the economic value of the SST must be one of the most disturbing aspects of the affair. This, after all, is a year in which airlines are already having to smile bravely at the sight of too many aircraft seats chasing too few passengers.

The issue on which the battle of the SST has been fought is exceedingly obscure. Although the present financial year is nearly three-quarters over, the budget for the Department of Transportation has not yet been settled, with the result that all the projects included in it are being provided with funds at a rate agreed by both houses of Congress in December last year. For the SST, this means a rate of \$210 million a year, not the \$290 million originally applied for. The question at issue in the past few weeks has been the rate of permissible expenditure during the fourth quarter of the year. In effect, the House of Representatives declared on March 18 that the rate of expenditure should be reduced to zero, and the question is whether the Senate will follow suit or, alternatively, allow the issue to stand over so as to fight it out again in a few weeks from now, when the budget for the next financial year will be ready for debate. One of the ironies of the situation is that the cost of cancelling the Administration's obligations to the manufacturers, and to the airlines who have made deposits, is reckoned to amount to \$119 million. To the end of the month, the total cost of the project seems to have been \$1,300 million, two-thirds of the amount being public money.

So why cancel? One member of the House of Representatives, Mr Silvio O. Conte (a Republican from Massachusetts), explained in the debate last week that he had been persuaded that there could be no serious threat to the environment from the operations of fleets of supersonic transport aircraft, but that there are good reasons why a project which is said to be economically sensible should be persuaded to stand on its own feet and not helped along with subsidies. Others have argued that



the cost of building the SST would inevitably price the aircraft out of the ordinary passenger market (and it seems to be accepted by all the committees concerned that Concorde will be equally at a serious disadvantage). As it turns out, the arguments in favour of the SST project have also usually been economic. Some emphasize that the project will provide work, others that sales of supersonic transports could contribute in important ways to the United States balance of payments and still others say that the project is a necessary way of keeping in the front of aeronautical engineering. To be sure, Senator William Proxmire, the sharpest thorn in the flesh of the SST, has been making a good deal of the increase of skin cancer which there may be from exposure to cosmic rays in flight, but it is a long time since even the optimists have allowed themselves to look beyond the construction of the two prototypes on which a decision to manufacture would be based.

#### NUCLEAR WASTE

### Internment in Kansas

THE continuing dispute about the proposal of the Atomic Energy Commission to bury radioactive waste in a disused salt mine near Lyons in Kansas reached the Joint Committee on Atomic Energy last week, when the committee nodded its grave head over the proposal in the new budget for the AEC, that money should now be spent on the acquisition of land for the permanent burial of cylinders of ceramic material containing substantial quantities of radioactivity. Under the arrangements now proposed, the Atomic Energy Commission would begin by shipping about three containers full of ceramic cylinders in 1976, and would increase the number of these shipments until something like 500 a year were being disposed of by the turn of the century. The radioactivity in the containers will be long lived, so that the half life of the arrangement will be of the order of a million years. At the hearings last week, as in the past, the Atomic Energy Commission's case (backed up towards the end of 1970 by a careful report from the National Academy of Science) is that enough experimental work has been done by now for there to be reasonable certainty that the activity will be safely contained. The AEC has been working on the site since the early sixties and, for what it is worth, the inhabitants of the nearest town would welcome some sign of industrial activity in the neighbourhood.

The case against the project, spelled out again last week by witnesses before the joint committee, is chiefly the re-

sponsibility of Dr William Hembledon of the University of Kansas, who has carried out for the state geological survey a study of the area in which nuclear wastes are to be buried. His criticism of the AEC proposal is so fierce that it has been answered point by point by no less a person than Dr Glen Seaborg, chairman of the AEC. At the hearings last week, the case presented was familiar enough, but it will be interesting to see how the joint committee manages to find a bridge between two quite opposing views. The state geologists argue that there are inadequate arrangements for carrying radioactive material to the site, that there are no arrangements for recovering material from the salt mine if something should go wrong and that the temperatures within the ceramic cylinders will be so great (1,800° C) that there are certain eventually to be occasions when cylinders break and release their active material into the strata underlying the salt mine. Perhaps recognizing that, in a head-on conflict, the AEC would probably win, the case against the burial ground was made to turn last week on the belief that more research and development is necessary if the disposal is to be properly controlled.

#### NORTH SLOPE

### No Oil Flows Yet

THE construction of the Alaskan pipeline, already more than one year late, seems now destined for a further spell in limbo. The first reaction of the Department of the Interior to a bruising public inquiry last month seems to have been to commission further studies of the problems of navigating tankers in the treacherous waters between Seattle and the southern terminal at Valdez, and to begin a search for alternative routes to that on which the consortium of North Slope petroleum companies has already spent \$300 million. At the same time, the Canadian Government has thrown a spanner in the works by suggesting that oil could be carried away from Alaska by running a pipeline 300 miles to the east, either along the coast or some distance offshore, and then turning inland to follow for a time the valley of the Mackenzie River.

If the Department of the Interior and conservationists in the United States are excited at this prospect, the oil companies themselves are cool to the point of indifference. They point out that several years of further exploration would be necessary before they could tell whether a Canadian pipeline would be practical, that the route is twice as long as the other, that it would be necessary to operate the inland

route in the strange never-never world of international taxation and that there is no assurance that the environmental problems would be any easier along the Canadian route than they are known to be in Alaska. At the same time, there is some doubt that the Canadian Government would be able to persuade those who have elected it to office that it is right and proper to import environmental problems which are apparently unacceptable in the United States. More should be known about this by the end of the week, when the oil companies with interests in Alaska will parade in Ottawa for further information.

The case against the Department of the Interior's description of the environmental problems of building the Alaskan pipeline was fully and even over-zealously deployed at a public inquiry last month. Most of the difficulties then experienced seemed to stem from the way in which the department laconically advised readers of its report that suitably careful operation of the pipeline would reduce "foreseeable environmental costs to acceptable levels". Given the department's careful listing of all the hazards to be avoided, however, it is inevitable that readers of its document should have been as alarmed as if they had been children told of all the dreadful things that would not happen to them if only they were able to behave correctly. In retrospect, however, the report may also have been injudicious in saying without investigation at first hand that the Canadian route would "serve mainly to shift the location of the ecological problems rather than cure them". The department's own assessment of the problem, the statutory document now known as the "environmental impact statement", also leads it on to say that a Canadian route would entail that the pipeline would be outside the direct control of the United States and that it would in any case be between two and four years before the route could be proved.

Possibly these tart comments have helped to move the Canadian Government to what appears to be a vigorous and unwonted demonstration that it is willing to help solve what is essentially an American problem. On the face of things, it will be hard for both countries to operate a pipeline system of this kind without coordinating their policies on the consumption of fuel in general and oil in particular.

The way things have turned out, the attention of the environmentalists seems to have been concentrated first on the possibility that a pipeline across Alaska would be broken by earthquakes and then on the danger that the off-loading operations at the southern end of the pipeline would pollute what is necessarily one of the wildest stretches of coastline in the world.

## NEWS AND VIEWS

## Superheavy Elements in Meteorites

THE mounting evidence for the presence of nuclei with atomic numbers of at least 92 in the cosmic radiation impinging on the Earth and the possible existence of the much discussed island of stability at mass numbers around 284 and atomic number about 110 have been an incentive to the continuing search for transuranic elements both in the cosmic radiation and in meteorites and lunar rocks. It now seems that there is very compelling evidence for the existence of superheavies in meteorites at some time in the past.

The techniques used in the two cases are, however, completely different; the identity of a charged cosmic ray nucleus has to be extracted from measurements of the amount of ionization it produces as it passes through either a stack of photographic emulsions whose grains are rendered developable by ionization or through a plastic detector in which the path of the particle can be etched chemically. On the other hand, any very heavy nuclei which were condensed out into meteorites some  $4.5 \times 10^9$  years ago would have quite a high probability of undergoing spontaneous fission into two or three fragments which are themselves still fairly heavy. This phenomenon can be studied by the rather elegant methods for detecting fission fragments or cosmic ray nuclei in certain clear minerals present in meteorites and lunar rocks. It is a rather careful study of the ionization trails of nuclei in such mineral samples which has allowed Bhandari *et al.* of the Tata Institute of Fundamental Research, Bombay (see page 219 of this issue of *Nature*), to state unequivocally that they have detected fission fragments from the breakup of elements with atomic numbers around 114.

Bhandari *et al.* have made use of the fact that in many non-conducting substances the ionization damage done by a charged particle can be made visible under a microscope by chemical etching. In the past, experimentalists using this technique have only been able to etch the tracks at a fractured crystal surface and the separation of the tracks caused by cosmic ray nuclei from those caused by the fission of an ultraheavy element is open to considerable criticism. But Bhandari *et al.* have succeeded in applying a technique which allows them to look at ionization tracks within the body of a suitable crystal where the number of cosmic ray tracks is severely depleted because of the surrounding material. The secret is to etch strongly a very long track or fissure which passes from the surface into the body of a crystal so that other tracks which cross the fissure are also made visible.

The length of the visible track of a charged particle in an extraterrestrial mineral is a fairly direct measure of the nuclear charge and energy of the particle and it is essentially on an examination of the distributions of measured track lengths that the establishment of the existence of superheavy elements depends. Bhandari *et al.* found that the most amenable tracks for this sort of measurement were those which were etched in the fissures (the so-called TINCLES—"tracks in the cleavage"). Remarkably, the track densities observed along such fissures were often much larger than expected theoretically, probably because of the movement of the

small number of plutonium, uranium and other heavy elements towards the flaw planes ("differentiation") during the initial condensation process. Instead of seeing about five tracks per 100  $\mu\text{m}$  of fissure, up to five hundred tracks per 100  $\mu\text{m}$  were often measured. In practice the upper limit of track density for which reasonable measurements could be made was about one hundred tracks per 100  $\mu\text{m}$ . (The existence of such a wide variety of track densities was in fact confirmed by inducing fission artificially within the meteorite samples with neutrons and examining the distribution of the tracks.)

It is possible to calculate the track lengths expected for fission products of plutonium and uranium isotopes as well as of superheavy elements and also to measure the track lengths of  $^{235}\text{U}$  fission products by neutron activation. Bhandari *et al.* found that the theoretical track lengths were short by about 2  $\mu\text{m}$  or so, chiefly because at the end of their range fragments do not have sufficient ionizing power to produce etchable tracks in the mineral. Basically, the track lengths seen in the meteorites can be divided into three groups. The 10 to 13  $\mu\text{m}$  division is thought to be caused by cosmic ray iron group nuclei and the 13 to 16  $\mu\text{m}$  range by heavier cosmic rays and uranium and plutonium fission products. The 16 to 22  $\mu\text{m}$  interval almost certainly contains tracks of fission products of the superheavy elements (in fact, these are probably greater than 18  $\mu\text{m}$  long, this being the upper limit for neutron induced fission tracks in  $^{235}\text{U}$ ). In the sample from the Angra dos Reis meteorite seven tracks longer than 18  $\mu\text{m}$  were discovered among 1,500 tracks and two of these were as long as 21.6 and 25.8  $\mu\text{m}$ . According to calculations for this sample, cosmic rays were responsible for fewer than 0.1 per cent of the tracks so it seems clear that these seven tracks were caused by fission of superheavy elements.

The cosmic ray contributions to the tracks visible in the other meteorite samples and in the Apollo rocks and "fines" could not, however, be ignored immediately but they were nonetheless shown not to affect the conclusion in any way. The proof of this lay in a comparison of the track length distributions for several different values of the very variable track density along the fissures for the TINCLES. As TINCLE densities increased, it was found that the proportion of the 13 to 16 and 16 to 22  $\mu\text{m}$  tracks relative to the 10 to 13  $\mu\text{m}$  group (cosmic ray iron group nuclei) also increased. In other words, this was additional support for the supposition that the uranium and other heavy nuclei were in fact sometimes concentrated at the fissures (possibly grain boundaries). Such excess fission tracks are also found in the Apollo "fines" but not in the Apollo rocks which are presumably too young to have contained very much fissionable material on solidification.

Now that the fission products of superheavy elements have been detected with a fair degree of certainty in meteorites, interest is bound to concentrate once again on the cosmic ray measurements at the Earth to see whether definitive proof of the existence of superheavy cosmic ray nuclei can be found.



## PROTEIN SYNTHESIS

**Chain Termination**

from our Cell Biology Correspondent

ANY lingering doubts about the universality of the genetic code have now been finally dispelled by Beaudet and Caskey (*Proc. US Nat. Acad. Sci.*, **68**, 619; 1971). They have now shown that the three codons UAA, UAG and UGA which serve as full stops, terminating the synthesis of proteins in bacteria, fulfil the same function in eukaryotic cells. Fifty-nine of the codons in eukaryotic messenger RNAs have now been assigned functions and in every case these assignments are identical to those for bacteria. In other words, bacteria and eukaryotes use identical genetic dictionaries.

Beaudet and Caskey have exploited the elegantly simple formylmethionine release assay, developed by Nirenberg's group, to prove that UAA, UAG and UGA act as termination signals in eukaryotes. In essence, the assay involves making a complex of a ribosome, from either a bacterial or eukaryotic cell, an initiator codon AUG, which serves as messenger, and a molecule of charged bacterial initiator transfer RNA, fmet-tRNA<sub>i</sub>. This complex acts as substrate and release factor(s) and the codon or tetranucleotide to be tested are added. If the tri or tetranucleotide contains a terminator codon, the fmet-tRNA<sub>i</sub> is hydrolysed as at termination and free formylmethionine is released and assayed.

Using ribosomes from rabbit reticulocytes and release factor from these cells, or from guinea-pig or Chinese hamster liver, Beaudet and Caskey found that the three tetranucleotides UAAA, UAGA and UGAA all stimulate the release of formylmethionine, whereas the tetranucleotides AAAA and UUUU containing lysine and phenylalanine codons do not. Furthermore, the trinucleotides UAA, UAG and UGA also fail to stimulate release of formylmethionine when rabbit ribosomes and mammalian release factor are used. By contrast when *Escherichia coli* ribosomes and release factors are used in this assay, either the tetranucleotides or the trinucleotide terminator codons stimulate release. This difference probably reflects the failure of the trinucleotides to bind to rabbit ribosomes.

The release factors in the bacterial and mammalian systems also differ. The bacterial factors have been resolved into three discrete proteins called R<sub>1</sub>, R<sub>2</sub> and S. R<sub>1</sub> apparently recognizes the two terminator codons UAA and UAG and R<sub>2</sub> recognizes UAA and UGA. Because both factors recognize UAA, and for other reasons, it seems likely that this codon (ochre) is probably the chief terminator whereas the other two, UAG (amber) and UGA, provide

accessory fail-safe signals to ensure that termination never fails. Factor S comes into the picture by stimulating the rate of release catalysed by either R<sub>1</sub> or R<sub>2</sub>. By contrast Beaudet and Caskey have failed so far to resolve mammalian release factor into components, and they have failed to find a stimulatory factor analogous to S. The mammalian factor stimulates release with, and must therefore recognize, all three terminator codons and with a molecular weight of about 255,000 it is a much larger protein, or complex of proteins, than the bacterial R and S factors, all of which weigh about 40–50,000 daltons. Whether it will ultimately prove possible to split the mammalian release factor into functional subunits remains to be seen.

Beaudet and Caskey also report that the addition of GTP to their assay stimulates the release of formylmethionine and the GTP is hydrolysed, whereas the GTP analogue GDPCP inhibits release. A mixture of ribosomes and release factor hydrolyses GTP and the tetranucleotide UAAA stimulates this reaction and also the binding of release factor to ribosomes. Certain antibiotics block the release of formylmethionine in the release assay but do not inhibit GTP hydrolysis. It might be speculated therefore that GTP plays a part in chain termination by promoting the binding of release factor to the ribosome (messenger) nascent polypeptide complex; the release factor might then act as a trigger and cause the peptidyl transferase enzyme of the ribosome to hydrolyse the peptidyl transfer RNA, instead of catalysing the formation of a further peptide bond.

Clearly when it comes to determining the precise interaction between ribo-

some, messenger and release factor it will be important to have pure release factor available, and while Beaudet and Caskey have been probing termination with mammalian release factor Klein and Cappechi (*J. Biol. Chem.*, **246**, 1055; 1971) have been purifying the bacterial R<sub>1</sub> and R<sub>2</sub> factors from the MRE 600 strain of *E. coli*. Each factor is a single polypeptide chain, R<sub>1</sub> has a molecular weight of 44,000 and R<sub>2</sub> 47,000, and if each has a spherical configuration the molecules must be about 47 Å in diameter. From their purification data, Klein and Cappechi estimate that there are 500 R<sub>1</sub> and 700 R<sub>2</sub> molecules per cell compared with about 30,000 ribosomes and an even larger number of elongation factor molecules per cell.

## ENZYMES

**Orbital Orbit**

from our Molecular Biology Correspondent

ONCE in a while one is treated to the awesome spectacle at the launching of a new concept, when, with bystanders cheering and band playing, it runs down the slipway and sinks without trace. Such, it now seems, was the fate of a theory of enzymatic catalysis propounded last year by Storm and Koshland. The hypothesis, to which they gave the euphonious name of "orbital steering", rested on measurements of reaction rates for  $\gamma$ -lactone formation in bicyclic compounds in which the position of the hydroxyl group was immobilized by the rigidity of the ring system. With the orbitals so aligned as to optimize the angle of attack, Storm and Koshland stated, rate increases of up to four orders of mag-

**Mixmaster Universe Straightened Out**

COSMOLOGISTS delight in constructing model universes, even if these models have little relation to the universe in which we live. One of the mathematical possibilities produced recently was a model in which particle horizons were removed, and information could be transmitted from one observer to any other observer before any given time. C. W. Misner, who put forward this model, christened it the "mixmaster universe", because this violation of the usual limits on causal interactions enables information to be spread throughout the whole universe and could (perhaps) have some bearing on the observed uniformity of the "3 K" background radiation, which has the same effective temperature irrespective of which part of the sky it is coming from.

Unfortunately for this idea, it now seems that the mathematical peculiarity

on which the model is founded has no valid application to our universe. M. A. H. MacCallum, of the University of Cambridge, presents in the next *Nature Physical Science* a discussion of the mixmaster universe problem which shows that the set of models which have no particle horizons is empty. It does still seem possible to remove the particle horizon in one direction only, at every time, but this set of models corresponds exactly with the set of rotationally symmetric models, which are already well known to cosmologists, and which even the simplest observations show to be invalid descriptions of our universe. MacCallum points out the prospect of obtaining more mathematical pleasure from this mixmaster universe problem by introducing quantum effects, a further intricate development which will have little bearing on practical astronomy.

nitude resulted, even after correction for the proximity (high local concentration) of the reacting groups, and they concluded that the angular sensitivity of such reactions was much greater than had previously been suspected.

Now such a radical new departure in organic chemistry could not go long unchallenged, and now Bruice, Brown and Harris (*Proc. US Nat. Acad. Sci.*, **68**, 658; 1971) seem to have savaged it beyond recovery. The proximity factor for the intramolecular reaction is easily calculable, and amounts to only 55.5. Thus the angular orientation effect of Storm and Koshland must account for many orders of magnitude in rate. Taking the area of contact favourable for reaction as a spot on a sphere of radius equal to the van der Waals separations, it transpires that the required rate enhancement would result from a change in orientation of only tenths of a degree, corresponding to a displacement of hundredths of 1 Å. Bruice *et al.* observe that the change of several kilocalories per mole in activation energy, which is what the enhancement amounts to, for an angular distortion of this size is incompatible with all that is known about chemical bonds: even in ethylene the torsional energy is only some 450 cal/degree, and indeed displacements orders of magnitude larger than those featuring in the orbital steering scheme are involved in the spectroscopically observed vibrational and rotational modes at ordinary temperature. These considerations will be the more applicable to the transition state. Bruice *et al.* reinforce their point with values of the energy change with torsional angle, based on molecular orbital calculations, for the transition complexes in  $\gamma$ -lactone formation. Their explanation for the enhanced rates lies in the greatly decreased entropy of activation which results from locking in the configuration of the reacting groups.

The phenomenon of enzymatic catalysis, which can lead to reaction rates as much as twenty orders of magnitude higher than can otherwise be achieved, therefore remains to mock the organic chemist. Bruice *et al.* favour a general explanation in terms of a reactant complex on the enzyme with a geometry close to that of the transition state. Reuben (*ibid.*, 563) offers some cogitations along similar lines. His concern is with the lifetime of the activated complex on the enzyme. When a small substrate molecule is attached to an enzyme, its tendency to separate from another reactant, with resolution of both species, is greatly diminished. The known "sticking times" for ligands on proteins are very long compared with rotational relaxation times of the small molecules themselves, and, as Reuben shows, the period during which the

reactants are immobilized in position for reaction is such that the reaction probability can, with a few assumptions, be increased by six to nine orders of magnitude. Other factors remain relevant, though often to an unassessable degree: desolvation on binding to the enzyme, so that effects of the solvation shell on the reaction are eliminated, configurational immobilization variously formulated (see, for example, Milstein and Cohen, *ibid.*, **67**, 1143; 1970) and favourable charge effects are all candidates for large contributions.

It may not have escaped the notice of particularly perceptive readers that in all the articles I have mentioned a few experiments provide a lot of mileage. The approach of the man of action is exemplified by the work of Klotz, Royer and Scarpa (*ibid.*, **68**, 262; 1971), who have synthesized a kind of proto-enzyme, by introducing

dodecyl groups, and imidazoles as side chains into a water-soluble polymer. The dodecyl groups serve to bind ester substrates, the hydrolysis of which is then catalysed by the imidazoles. An acyl intermediate seems to be formed, just as in chymotrypsin for example, and the rates for hydrolysis of uncharged nitrophenyl esters are more than two orders of magnitude up on those catalysed by imidazole alone. The design of this experiment may recall the work of Morawetz and his colleagues on the catalysis of ionic reactions by polyelectrolytes of opposite charge to the ions. The reactants concentrate in the counterion layer of the polyelectrolyte, and rate enhancements of five orders of magnitude have been observed. These and other elegant experiments have been reviewed in a captivating article by Morawetz (*Acc. Chem. Res.*, **3**, 354; 1970).

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## The Start of Influenza Virus RNAs

WITH so many bacterial and bacterial virus RNAs under their belts, biochemists wedded to the business of determining the sequences of nucleic acids are now turning their attention to animal viruses. For example, in next Wednesday's *Nature New Biology*, Young and Content report an analysis of the first nucleotide at the beginning of the handful of RNA molecules which comprise the genome of influenza virus.

Influenza virus has a curious genome; it seems to be made up of five or six distinct species of RNA and this no doubt accounts for some of the unusual genetic properties of the virus. The very high rate of recombination and the consequent misery of 'flu epidemics probably result from an exchange of one or more of these RNAs between different strains of the virus replicating in one cell. And the so-called von Magnus phenomenon—the decrease in the size of the viral genome as the virus is repeatedly passaged at high multiplicities in cells—no doubt results from the loss of one or more of these RNA molecules.

But the fact that 'flu virus genomes are made of several RNA molecules rather than a single molecule poses as many questions as it solves. For example, are these RNAs generated by cleaving one very large molecule into the appropriate number of smaller pieces, how closely related are the pieces and precisely how many of each are present in the genome? Answers to some of these questions may await an analysis of the complete sequences of the molecules, but by determining the nature of the very first base at the beginning of the 'flu virus RNAs, Young and Content have answered the first.

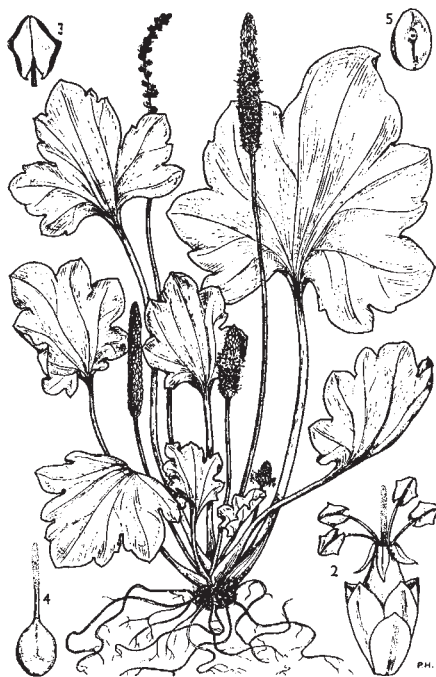
Using the analytical procedures which have been developed for determining the nature of the first base of bacteriophage and plant virus RNAs, they have shown quite convincingly that the RNA molecules which together form the 'flu virus genome all begin with an adenine nucleotide with four phosphate groups; in other words the sequence reads pppApX-. This finding closely parallels that obtained with various RNA bacteriophage RNAs which begin pppGpX, and it has some interesting implications for speculation about how the 'flu genome replicates. Because the first nucleotide is pppA in all the 'flu RNAs it seems likely that they are all replicated individually and that they do not arise from the cleavage of a single very large RNA chain. If that is the case at least some of the RNAs should begin either with an unphosphorylated base or a base with only one phosphate residue, pApX or ApX. A small RNA molecule generated by cleavage from some internal region of a larger RNA molecule would not have three phosphate groups attached to its first base.

Young and Content have also found that the number of terminal pppAp residues is more than would be expected if the 'flu virus genome is assumed to be made of only five or six RNA chains. This suggests that the genome is in reality even more heterogeneous than has hitherto been suggested. As they point out, it may be that each virus particle contains only five or six sorts of RNA but that more than one copy of one or more of these different RNAs is present or, of course, a few small, partially synthesized RNA chains may be incorporated as the virus particles mature. Questions such as these will no doubt soon be answered.



## FLOWERING PLANTS

## East African Flora



Descriptions of eleven more families of flowering plants have been added recently to the *Flora of Tropical East Africa* which is being prepared by the staff of the Royal Botanic Gardens, Kew, for the East African Community (parts are obtainable from government bookshops in the UK and in East Africa). This illustration is taken from *Plantaginaceae* by B. Verdcourt (price 15p) and shows the plantain *Plantago palmata*, which is found in montane forest in East Africa, often by streams or on boggy ground. (1) Habit; (2) flower; (3) anther; (4) young fruit; and (5) seed.

## SPACE BIOLOGY

## Biological Recycling

from our Photosynthesis Correspondent

It may soon be possible to use photosynthetic organisms in manned space vehicles and stations to regenerate food, pure water and oxygen from human excretion and exhalation. The design of an optimal environment and selection of ideal organisms for this purpose was the subject of a meeting held in London by the Royal Society on March 11.

Intact higher plants were initially considered as possible candidates. In fact, as Professor F. R. Whitley (King's College London) pointed out, higher plants do have a considerable nutritional potential and could be used to convert waste nitrogen products of animal origin into first class leaf protein for redigestion. Higher plants also have the ability to recycle important inorganic salts, including essential elements such as coal and iodine.

There was a great deal of discussion as to the various ways in which suitable

plants could be made more efficient in their ability to utilize light energy to drive their various photosynthetic reactions. It was generally agreed that levels of carbon dioxide higher than those usually found in the Earth's atmosphere, which could readily be produced in the closed environment of a space station, would increase considerably the rate at which plants evolve oxygen and fix carbon dioxide. Suggestions as to the type of plant most suitable for this ranged from Chinese cabbage and chicory to tropical grasses such as sugar cane. Nevertheless, there are many disadvantages in using higher plants in this type of project such as the large leaf area required per man per day—as much as 8 m<sup>2</sup>—and their long vegetative phase of growth.

A much more satisfactory biological system for creating a microcosm in which regeneration of food and oxygen could be complete is likely to involve the use of cultures of photosynthetic microorganisms. Professor G. E. Fogg (Westfield College London) argued in favour of using a unicellular green alga such as *Chlorella*. Combination of cultures of this alga and anaerobic bacteria could be used to recycle waste products as well as fixing carbon dioxide and generating oxygen. From experiments with animals, cultures of *Chlorella* can certainly supply sufficient oxygen to maintain air in a closed system

in a state fit for breathing, and are also a reasonable nutritional source, although over long periods of time there would be a need to supplement the algal diet with other types of food. Professor Fogg emphasized, however, that there would be many difficulties in developing a highly organized ecosystem of the type he envisaged. Dr. A. R. Crofts (University of Bristol) also shared the view that microorganisms were suitable as recycling agents. He extended the idea one step further by introducing a third type of organism—photosynthetic bacteria. He argued that these organisms had the additional advantage of utilizing far-red light to convert partially reduced carbon compounds, normally obtained from anaerobic bacterial action on sewage, to more highly reduced compounds suitable as food for the animals in the closed system.

Whether photosynthetic organisms can effectively compete with miniaturized nuclear energy sources or improved photocells as a way of supplying energy to the astronaut faced with a long period in a closed space vehicle or station did not clearly emerge from this meeting. There does seem, however, every reason to believe that cultures of photosynthetic algae and bacteria could be used to convert human excretion into organic food material as well as producing satisfactory amounts of oxygen and pure water.

## Gravity Waves may Set the Earth Ringing

SEARCHING for confirmatory evidence of Weber's gravity waves is becoming a fashionable exercise. In next Monday's *Nature Physical Science*, V. S. Tuman of Stanislaus State College, California, puts forward, in a tentative manner, the notion that excitation of the Earth by gravity waves may be the explanation of some anomalous results obtained with gravity meters. Tuman has already described in *Nature* a cryogenic gravity meter developed in cooperation with Stanford University to record the oscillations of the Earth (229, 618; 1971), but the anomalous effect has also been noticed in records taken elsewhere. What it amounts to, in brief, is that as a rule the even harmonics of the eigen vibrations of the Earth detected by the gravity meters contain more energy than the odd harmonics.

Tuman points out that the existence of the effect is not definite, however. But two series of observations out of three carried out with the cryogenic gravity meter show the discrepancy in energy content between the odd and even harmonics, and the effect is also present in results reported by Block *et al.* (*Nature*, 226, 343; 1970). One explanation considered and then discarded by Tuman is that the oscillations

in question are excited by earthquakes occurring at locations which ensure that the gravity meter at Stanislaus College is always at a node corresponding to no motion for the odd harmonics and a finite motion for the even harmonics.

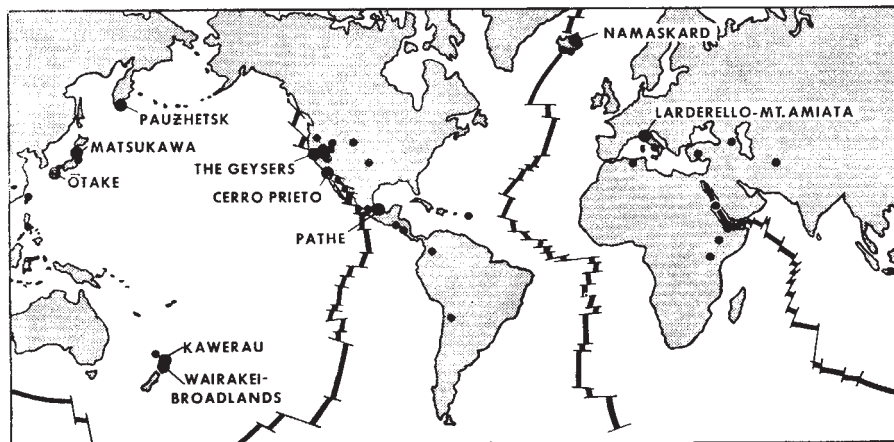
This could be the explanation if the earthquakes exciting the vibrations were at an angular distance from the gravity meter of fairly precisely 90°. But the explanation fails to work if the earthquake is more than a few degrees off this orientation. With earthquakes at least of Richter magnitude 5.5 being necessary to generate detectable oscillations, and for other reasons, this mechanism is not particularly convincing.

Tuman suggests therefore that the anomalous distribution of energy might have an explanation from outside the Earth, in gravity waves of high energy density. Such a gravity wave pulse would be expected to couple energy into the  ${}_0S_{2n}$  modes and their overtones with quadrupole moments, leaving the odd spheroidal modes ( ${}_0S_{2n+1}$ ) unaffected. The evidence for this mechanism is still far from convincing, however, but Tuman points out that a network of similar gravity meters would help to solve the problem.

## GEOTHERMAL POWER

# Harnessing Hot Springs Around the World

from our Geomagnetism Correspondent



Geothermal areas of the world. Large circles, producing fields; small circles, promising areas.

THE most energetic process taking place in the Earth today is the outward flow of heat through the Earth's surface. This happens everywhere, but certain regions have higher heat flows than others and some are potentially capable of supplying heat commercially. The nice thing about geothermal power is its cleanliness—the severity of pollution is much reduced compared with fossil fuels; but this is not to say there are no pollution problems at all. In the Salton Sea area of California, for example, where natural hot brines are used in heating systems the mineral content of the brine, about a quarter of all the dissolved salts, poses its own problems of waste disposal.

Hot springs have been used for baths ever since Roman times at least, though the use of natural steam for the manufacture of electricity did not begin until early in the present century. The first commercial use of the Earth's heat took place at Larderello, Italy, in 1777 when borax was recovered from natural steam and hot water vents—a product which is still found there today. In 1905, the world's first power generating station using natural steam energy was also established at Larderello. Iceland seems to have been the next place to develop geothermal energy for domestic and commercial purposes, for hot water energy was used there for space heating in 1925. In that year attempts were also made to harness geothermal steam in New Zealand, although no serious developments took place there until 1946 when the New Zealand government took over development of natural steam.

The world production of power from the Earth's natural heat now exceeds

one million kilowatts, though even under current economic conditions this could probably be increased ten-fold. Larderello still leads in production (400,000 kW), followed by New Zealand (more than 170,000 kW) and the United States (83,000 kW). And many other countries—for example, Algeria, Chile, Czechoslovakia, Hungary, China, France, Turkey, Kenya and the Soviet Union—are either searching for geothermal sources or are looking at how existing sources might be harnessed. In Japan, in particular, geothermal energy development has a high priority in national planning.

Geothermal resources in the United States were first investigated during the early 1920s, though the first commercial wells were not drilled until 1955 in The

Geysers area, 75 miles north of San Francisco. Four wells drilled to depths of less than 1,000 feet began producing power in 1960 at the rate of about 12,500 kW, though this capacity has since increased to 83,000 kW. In the meantime, on December 24, 1970, Congress passed the Geothermal Steam Act which requires the Department of the Interior to list all land with geothermal resources as a first step to their development. The US Geological Survey has so far identified 1.3 million acres of land, primarily in the west, as being potentially suitable for geothermal development, the estimated steam reserves being equivalent to about 30,000 mW of electric power, or about 5 to 10 per cent of the power to be derived from fossil fuels in the same area.

## EVOLUTION

## What Use is Sex?

from our Population Genetics Correspondent

IN his classic book *The Genetical Theory of Natural Selection* published in 1930, Sir Ronald Fisher briefly discussed what advantage is to be gained from sexual, rather than asexual, reproduction. Recently, this has been a controversial issue, and now Maynard Smith, in an article in the *Journal of Theoretical Biology* (30, 319; 1971) entitled "What Use is Sex?", describes computer simulations which essentially confirm Fisher's original conclusions.

Unfortunately, in the manner which was typical of him, Fisher never fully explained how he arrived at his conclusions. But his argument is basically as follows. If two advantageous mutations occur, they can only be incorporated into the same individual if sexual reproduction is available to bring the mutations together by the process of

## Spinodal Behaviour in an Alkali Feldspar

SPINODAL decomposition has been observed in an alkali feldspar (Na,K)Al Si<sub>3</sub>O<sub>8</sub>, a common rock forming mineral, following heat treatment in the laboratory. In next Monday's *Nature Physical Science*, Owen and McConnell report that they have seen modulated structures using transmission electron microscopy and they show an example with a wavelength of 160 Å.

Similar structures are well known in metal, glass and oxide systems (C. Hilliard, *Phase Transformations*, ASM, 497; 1970) and there have been several observations of modulated structures in natural feldspar and pyroxenes (Ca, Se,Mg)Si O<sub>3</sub>. But this is the first published report of the production of a modulated structure in a mineral under

controlled hydrothermal conditions.

Although the existence of spinodal decomposition has been inferred in many cases from the observations of modulated structures and the associated satellite reflexion in the fraction pattern, such structures could be produced by other mechanisms (for example, elastic energy or diffusion effects). Spinodal decomposition is associated with a negative diffusion coefficient and a specific variation in the modulation wavelength and amplitude with time and with temperature (J. W. Cahn, *Trans. AIME*, 242, 166; 1968). Confirmation of a spinodal mechanism has only been achieved by quantitative small-angle X-ray scattering data in a very few systems.



genetic recombination. If reproduction is entirely asexual, the mutations will only be brought together after one of the mutations has spread through the population so that the second mutation then has a good chance of arising in individuals carrying the first. Fisher said that the rates of adaptation among sexual and asexual groups would depend on the number of genetic loci at which favourable mutations could arise. The greater the number of loci, the more rapid would be the evolution of a sexually reproducing species compared with an asexually reproducing one. Fisher did not explain exactly how he reached this conclusion.

Crow and Kimura (*Amer. Nat.*, **99**, 439; 1965) showed mathematically that if favourable mutations are single events, of which there are always several at any one time, then indeed sexual reproduction will produce much faster evolution. Maynard Smith, however, then showed (*Amer. Nat.*, **102**, 927; 1968) that if, on the other hand, mutations are recurrent events the speed of evolution will be the same in both sexual and asexual species. To prove this, however, he assumed that if there are two favourable mutations, there will always be some individuals who carry both of them. It was pointed out that this cannot be true if the mutations are initially rare. If they are each at a frequency of  $10^{-6}$ , individuals carrying both mutations would be at a frequency of  $10^{-12}$  and so could never be present in a finite population.

In his more recent article, Maynard Smith considers the case of ten loci at each of which favourable mutations occur. Assuming that the selective advantage is the same at all the loci, he calculates the number of generations required for the average number of favourable mutations to reach 9.9 (out of a possible 10) under asexual and sexual reproduction. For a population of  $10^7$  individuals, asexual reproduction took about 1.6 times as many generations as sexual reproduction to get to the same point. And for a population of  $10^8$ , asexual reproduction took about 5.8 times longer than sexual reproduction. In populations of  $10^6$ , the speed of evolution by sexual and asexual reproduction was about the same. This is because in the smaller populations the favourable mutations take much longer to become established than they do to spread through the population. Thus each favourable mutation which became established would usually have spread through the population before the next favourable mutation establishes itself. Sexual reproduction has no advantage because at any one time there are not enough different favourable mutations for advantageous combinations to be formed in the sexual process. In the larger populations the time for a muta-

tion to spread is much longer than the time for it to become established and sexual reproduction produces quicker evolution because advantageous combinations of mutations can be formed. In these conditions, when evolution is almost complete, Maynard Smith shows that the ratio of the time of evolution by asexual reproduction to its time by sexual reproduction is roughly equal to the number of loci as originally suggested by Fisher.

These are the long term benefits of sex. But Maynard Smith shows there may be immediate advantages in sexual reproduction if the environment is changing rapidly. His analysis shows that if one combination of genes is favourable in one generation and another combination is favourable in the next, then sexual reproduction will be advantageous because it will promote the formation of the alternative combinations in the successive generations. Such reversals of selective advantage are perhaps unlikely, and so it seems that Fisher's original analysis of the advantage to be gained from sexual reproduction provides the best explanation of the evolution of sex.

## NATURAL DISASTERS

### Tornado Toll

from our Geomagnetism Correspondent

THE history of earth science is closely linked with the history of natural disasters; but in spite of the great advances made in the former in recent years, natural disasters continue to happen with monotonous regularity. Consider tornadoes in the United States, for example. During 1970 no less than 650 tornadoes struck this country killing seventy-three people and producing untold millions of dollars worth of property damage. Texas, Oklahoma and Florida were the worst hit with 121, 50 and 46 tornadoes respectively, whereas Alaska, Delaware, Hawaii, Idaho, Maryland, Rhode Island and Wyoming had none at all.

With the improvement of warning systems the annual death toll has, however, decreased dramatically over the years, but the damage to property has shot up equally spectacularly. From 1930 to 1949, for example, there were on average 222 deaths a year compared with an average of 118 deaths per year

### Riser Whistlers at a Ground-based Station

IN next Monday's *Nature Physical Science*, S. K. Dikshit *et al.*, from the Banaras Hindu University, India, report the detection of "riser" whistlers at a low latitude ground-based observing station. Whistlers are natural audio-frequency electromagnetic waves and have their origin in lightning flashes. They are named on account of the characteristic sound they produce in a high gain amplifier connected to a suitable receiving antenna—normally a sound (whistle) of descending frequency having a duration of the order of one second. They were first heard on telephone lines in Austria in 1886 and were a considerable nuisance to Barkhausen in the First World War when he was attempting to listen in to the telephone communications of the allies. Often the number and intensity of whistlers were so great that no other sounds could be heard in his equipment. At that time the origin of the sounds was unknown.

The British radio scientist Eckersley studied the dispersion of electromagnetic signals in the ionosphere and deduced a theoretical expression for the decrease with time of the received frequency of the signal radiated by an impulsive source. The theory involved the study of the propagation of electromagnetic waves in the ionospheric plasma and fitted some experimental observations well. It was not until 1953, however, that Storey at Cambridge produced a theory that is the basis of present under-

standing of the phenomenon (*Phil. Trans. Roy. Soc., A*, **246**; 1953). Storey suggested that the very great dispersion observed in whistlers was produced by the propagation of electromagnetic energy through the ionosphere. He showed that at audiofrequencies the wave energy could be guided along the magnetic field lines of the Earth and thus travel great distances into space before returning to the Earth and re-penetrating the ionosphere to be observed on the ground as a whistler. Thus the whistler is now understood to arise from a peculiar property of the refractive index of an ionized plasma when permeated by a magnetic field. Ionospheric whistlers are related to the "helicon" mode waves of the solid state physicist.

The latest Indian work, however, deals with a rather rare type of whistler—the riser, in which the frequency of the observed tone increases with time. There are few previous observations of such risers at ground-based stations and none has previously been reported from such a low latitude. Risers are frequently observed by satellite-borne receivers but hardly ever seem to penetrate the ionosphere to be seen on the ground. The Indian scientists explain their observations in terms of the effects of ionospheric heavy ions on the wave propagation. Their results will doubtless provoke further discussion of the complex phenomena involved.

during the period 1950–1970. But the average property toll has now risen to more than \$50 million per annum, an annual loss reached only twice before 1965 (in 1953 and 1957); and it is easy to see why. In Florida, for example, only a few years ago tornadoes would cut wide paths but encounter little more than sand and scrub. Now the same region is likely to contain tightly packed housing, commercial properties, trailer parks and numerous other trappings of civilization—so tightly packed, in fact, that the tornado which struck Titusville on March 5 last caused damage of more than \$1 million in only 1.9 miles. Actually, the total damage to property in 1970 was several times the average trend chiefly because of the \$135 million toll from the Lubbock, Texas, tornado of May 11, 1970.

The other natural disaster to which the United States is particularly prone is, of course, the earthquake; and 1970 was a bumper, but deathless, year for those too. A total of 238 quakes was felt in fourteen states; but this was the fifth, though final, successive year with no fatalities. Before the southern California earthquake of February 9, 1971, which killed sixty-four people, the most recent deaths from earthquakes were the seven in Seattle in 1965. In 1970, however, California led the stakes with 130 earthquakes. Alaska came second, as usual, with sixty-eight; but in view of the thinly populated areas there many quakes probably went unrecorded or unfelt.

## VENUS

### Surface Properties

from a Correspondent

AFTER all the ballyhoo over the Apollo programme it is encouraging to learn that probing the solar system with a ground-based radio telescope can still yield valuable information which is complementary to spacecraft experiments. Such is the case with Venus, where, in spite of the great strides in knowledge of the planet's upper atmosphere obtained by the Mariner and Venus probes, understanding of its surface remains obscure. This region can, however, be explored by radar and radio techniques, and recently two Cambridge astronomers, R. W. Hall and N. J. B. A. Branson, have published a high resolution map of the 6 cm radio emission from Venus (*Mon. Not. Roy. Astron. Soc.*, **151**, 185; 1971).

At a wavelength of 6 cm, most of the radiation seems to come from the Venus atmosphere, and the degree of linear polarization is less than 1 per cent. Hall and Branson have dovetailed their new radio results with temperature and pressure profiles of the upper atmosphere

obtained by Venera V and VI. Adiabatic gas models can be fitted to the 6 cm brightness temperature, and the Venera data are used as a guide to the atmospheric parameters at a height of 20 km.

The best model atmosphere consistent with all the observations has a surface pressure of  $110 \pm 15$  atmospheres, a surface temperature of  $790 \pm 20$  K, and a water vapour content of  $0.4 \pm 0.3$  per cent. Hall and Branson also tried isothermal models of the lower atmosphere but these give too low a brightness temperature at 6 cm. These workers further claim that there can be no large scale dust clouds present in the Cytherean atmosphere.

An enigmatic feature in the high resolution 6 cm map is a bright emis-

sion region 50 K hotter than the rest of the planet. The excess is too large for it to have an atmospheric origin, and it may indicate a hot spot on the surface. Nevertheless, it does not show up at lower frequencies, and so its true nature remains obscure.

## LORIKEETS

### Pollen Harvesting

FOR more than a hundred years, the brush tipped tongue of lorikeets, a group of birds in the parrot family, has been thought to be an adaptation for extracting nectar from *Eucalyptus* flowers and other tree blossoms.

### Site of Action of Sex Hormones

THERE seem to be specific binding sites for female sex hormones on the membranes inside male rat liver cells, and specific binding sites for male sex hormones on the membranes inside female rat liver cells. And the act of binding by the "opposite" sex hormone seems to be a precondition for the arrival of polysomes at the interior membranes of liver cells and, hence, the participation of the membranes in protein synthesis. The finding is not only an unexpected twist to the story of sexual differentiation: it also brings a fresh perspective to the vexed question of the locus of action of steroid hormones.

In two articles in next Wednesday's *Nature New Biology*, B. R. Rabin and his colleagues at University College London describe how they monitored the binding of polysomes to isolated samples of liver membranes by an indirect but ingenious technique based on a membrane-borne disulphide interchange enzyme. Rabin and his group have been exploiting the fortuitously useful characteristics of this enzyme for some years now: it probably has a hand in the synthesis of proteins (such as insulin and ribonuclease) which contain several disulphide bridges in their structure, and its activity can be followed fairly straightforwardly by exposing it to an inactive form of the enzyme ribonuclease in which the disulphide bridges are randomly cross-linked, and measuring the rate of reappearance of the ribonuclease's catalytic activity (Williams, Gurari and Rabin, *FEBS Lett.*, **2**, 133; 1968).

What makes the enzyme really useful is the fact that it is situated in the membrane in or near the binding sites for ribosomes. As ribosomes or polysomes bind, they mask the enzyme's catalytic activity, and the fall in activity is directly proportional to the extent of

ribosomes binding, as independently measured by electron microscopy or RNA assay. And using the enzyme in this way, Rabin *et al.* have now shown that "smooth" membranes from rat liver cells (lacking bound ribosomes *in vivo*) can be persuaded to take up ribosomes if the female sex hormone oestradiol is added to the male membrane preparation, and the male sex hormone testosterone to the female. Simple calculation suggests that one molecule of steroid is sufficient to attach one ribosome.

And conversely, the carcinogen aflatoxin B<sub>1</sub> strips "rough" membranes of their *in vivo* complement of bound ribosomes (Williams and Rabin, *FEBS Lett.*, **4**, 103; 1969), and oestradiol protects male membranes against this stripping action, and testosterone protects female membranes. The "appropriate" sex hormones have essentially no action in these experiments. Rabin *et al.* have also measured directly the binding of labelled steroids to "smooth" membrane fractions from rat liver: the same pattern emerges. Membranes of each sex seem to have separate tight binding sites for testosterone and oestradiol, but female membranes bind more of the former than the latter, and vice versa. Of course the membranes may come out of the rat nearly saturated with their own sex hormone, and the group is at present investigating this possibility. But it is very surprising that the membranes should have such a marked affinity for the other sex hormone of the pair, an affinity that seems certain to have functional consequences in terms of the cell's pattern of protein synthesis. For there is considerable evidence that polysomes bound to the cell's inner membranes differ markedly from those floating free in the cytoplasm in their protein output.



Indeed, one author has stated that lorikeets "literally gorge" themselves with nectar. It now looks, however, as though the association of brush tongued lorikeets with nectar gathering is not as important as was once thought, because two Australian workers, D. M. Churchill and P. Christensen, have found that one lorikeet, at least, *Glossopsitta porphyrocephala*, the purple crowned lorikeet, feeds primarily on pollen which it extracts from the flowers of the karri (*Eucalyptus diversicolor*) high up in the canopy 100 to 200 feet above ground (*Aust. J. Zool.*, **18**, 427; 1970). Indeed, the volume of nectar flow of the karri flower seems to be too sporadic and too small to provide alone a sufficient source of energy for a bird the size of the purple crowned lorikeet, which is about 50 g weight. But when the flowers are in full bloom and there is an accompanying nectar flow, the bird does take nectar in and it seems to supplement the diet of pollen in some way. It is possible that the sugars present in the nectar—sucrose, glucose, fructose and melibiose—are absorbed from the crop, and Churchill and Christensen suggest that these sugars may provide the principal substrate from which the subcutaneous fat in the lorikeet is synthesized. The pollen, nevertheless, seems to be essential for the bird's metabolism.

Churchill and Christensen have calculated that the daily calorie requirement of a bird the size of a purple crowned lorikeet is about 8.0 kcal. In theory, this amount could be supplied by 8.1 g or 8.9 cm<sup>3</sup> by volume of nectar. During a period of maximum flow of nectar the bird could obtain this volume by visiting 297 flowers in daylight, but this amount of flow is the exception rather than the rule. Normally, the calyx of the karri flower is only one-tenth filled with nectar, so to obtain the required volume the bird would have to visit 2,970 flowers in 12 hours at the rate of one flower every 14 s. It would therefore seem a physical impossibility for the bird to find time to visit enough flowers in a day to satisfy its basic energy requirements, particularly when it is remembered that karri flowers do not produce nectar until they are in full bloom after the dry months at the beginning of the year.

On the other hand, the amount of pollen necessary to provide 8.0 kcal is 4.8 g. The weight of pollen produced per karri flower is 0.01 g, and it follows that the purple crowned lorikeet must visit 480 flowers during the 12 hours of daylight at the rate of one flower every 90 s to obtain this amount of pollen. According to Churchill and Christensen, it takes pollen from forty-three flowers to fill the bird's gut completely, so that during a 12 h day the gut needs to be

filled eleven times. This provides a rate of passage of pollen through the gut of approximately once every hour. It seems therefore that the purple crowned lorikeet must spend most of its day in search of karri pollen grains. The way it harvests these dust-like grains in the million during the driest months of the year when there is no flow of nectar in the karri flowers is interesting. Churchill and Christensen found that the bird is able to remove

the pollen grains without detaching more than 1 per cent of the anthers which contain the pollen. Each grain measures 37.5  $\mu$ m by 22  $\mu$ m along the sides and from pole to pole respectively, and Churchill and Christensen suggest that the depression behind the papillae on the lorikeet tongue, and the unguis, a horny collar which flanks and underlies the depression, play a part in pressing the dry grains into a wet cohesive ball before it is swallowed.

## Lipids and Sodium Transport across Cell Membranes

It is now generally accepted that the maintenance of optimum gradients of sodium and potassium ions across the cell membrane is mediated by an enzyme system, first discovered by J. C. Skou in 1957, which constitutes an integral part of the structure of the membrane. The system can be demonstrated in fragments of membrane prepared after disruption of the cell structure and hydrolyses ATP in a unique reaction requiring both sodium and potassium ions in the medium for its activity. In the intact cell the energy liberated by the reaction is used to pump sodium ions out in exchange for the entry of potassium. It has been recognized for some time that this transport enzyme system is highly complex, and something is now known of its mechanism of action. Basically, this seems to involve, first, a sodium-dependent transfer of the  $\gamma$ -phosphate of ATP to a carboxyl group located in a membrane protein to form an acyl phosphate, followed by a potassium-dependent hydrolysis of this bond to liberate inorganic phosphate. The exact mechanism by which this hydrolytic process moves the cations across the membrane is unknown, but conformational changes in protein structure and allosteric effects are most likely concerned. The system probably comprises two or more proteins, one of which may act as an ion carrier as a result of a change in conformation induced by its phosphorylation.

A particularly controversial aspect of the complexity of the transport system concerns the extent to which its functioning is dependent on lipid components in the membrane. Removal of membrane lipids by a variety of methods has invariably inactivated the enzyme system, but its reactivation by adding back various lipids has proved more of a problem. It has therefore been difficult to decide whether the removal of lipid is having a non-specific inactivating effect, perhaps by disorganizing the precise orientation of the protein components of the system, or whether an individual lipid is intimately involved in the transport pro-

cess. Evidence for the latter concept comes from the work of Wheeler and Whittam (*J. Physiol.*, **207**, 303; 1970), who inactivated the system by separating the bulk of the lipids from a membrane preparation by deoxycholate and then found that they could recover sodium and potassium-dependent activity by adding a dispersion of phosphatidylserine; other phospholipids were ineffective and the suggestion was made that the transport system involves a complex unit of phosphatidylserine and the protein of the ATP-hydrolysing enzyme(s). In next Wednesday's *Nature New Biology*, T. Noguchi and S. Freed describe another experimental approach which has led to a rather different conclusion. These workers delipidated their tissue preparation by treating it with a mixture of chloroform and methanol at  $-75^{\circ}$  C and separating the soluble lipids by filtration at this temperature. Enzyme activity in the inactive protein residue was successfully restored by adding back the chloroform-methanol extract at  $-75^{\circ}$  C and removing the solvent by molecular distillation at the same low temperature. When the various lipids present in the extract were examined individually for their ability to restore activity, only cholesterol was found to be effective.

It is important to note that Wheeler and Whittam only recovered by the addition of phosphatidylserine a small fraction of the total activity present in their tissue preparation before treatment with detergent, whereas Noguchi and Freed found that cholesterol alone restored fully the activity present before delipidation. The effectiveness of chloroform-methanol mixtures at  $-75^{\circ}$  C as delipidating agents is, however, an open question, and it is possible that at this very low temperature the solvents are more efficacious in removing cholesterol from the membrane than the more polar phosphatidylserine molecule. The two sets of results are not therefore necessarily incompatible, and it now seems possible that lipids may be involved in more than one way in sodium transport across membranes.

# European Geophysical Community?

PETER J. SMITH

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**Next week, the first European Earth and Planetary Physics Colloquium will take place in Reading, England. Here the colloquium's Scientific Secretary gives a personal view of the history, philosophy and future of the project.**

It is appropriate that two of the participants in the first European Earth and Planetary Physics Colloquium will be Homer E. Newell and Athelstan F. Spilhaus, jun., president and executive director, respectively, of the American Geophysical Union (AGU). For ever since the basic idea of an annual meeting of European geophysicists was conceived by Keith Runcorn and a few others, those of us responsible for its organization have regarded the AGU as both a model and a source of inspiration. Indeed, when the whole matter was first discussed in public in Madrid eighteen months ago the use of the working title "European Geophysical Union" left the participants in that first exploratory meeting in no doubt about the parentage of the proposed European organization; and in turn it was made quite clear to the conveners of that meeting that, without wishing to fall too heavily for a carbon-copy philosophy, the time was ripe for the adoption and adaption of some of the AGU's more successful techniques in Europe. Only a small proportion of European geophysicists may ever have attended the annual meeting of the AGU in Washington; but it is clear that those who have hold it in high regard.

It was perhaps inevitable that some of the basic concepts discussed in Madrid should change during the succeeding months. For one thing, the title "European Geophysical Union" was clearly a misnomer in the European context—the AGU is, of course, a union in the strict sense of the word. The union thus soon became a society; but even that quickly became too ambitious without the certainty of widespread support throughout Europe. In the light of harsh reality the original enthusiasm was therefore crystallized into the plans for the first of what is hoped, but not guaranteed, to become a series of annual meetings. The Royal Society, in particular, was apparently unhappy at the idea of launching a society—and the administrative set-up it would imply—before the need and support for a more intimate relationship between European geophysicists had been amply demonstrated; but kindly agreed to a sponsoring loan for the limited purpose of a meeting in England. In retrospect, this was a wise constraint. Next week's colloquium is thus a simple experiment which may or may not lead to better things.

## The Philosophy

What is it then about the American Geophysical Union, and in particular its annual meeting, that we hope to emulate in Reading next week? The first thing is simply the value of bringing together in one place as many as possible of the geophysicists of Europe. Of course, in this day of what is coming to be regarded as the unhealthy and time-wasting proliferation of national and international meetings, this basic purpose of the conference is in danger of being devalued and

as a result is often regarded with a certain amount of cynicism. But in spite of the distaste frequently expressed on this score, it may come as a surprise to learn that there is not, and never has been, an opportunity for European geophysicists to congregate together. To be sure, there is the International Union of Geodesy and Geophysics which holds meetings every few years and whose member societies hold separate assemblies from time to time. But though not wishing to deny the value of these large international gatherings, it seems to me that there is also a case to be made for smaller, more frequent and more informal European meetings, away from the international glare, at which European geophysicists can exchange information and ideas on topics of direct interest to them without becoming absurdly parochial. The first European Earth and Planetary Physics Colloquium is thus predicated on the assumption of this need and will presumably stand or fall by its validity.

It is tempting to see in the colloquium a partial solution to problems which affect European geophysicists but whose origins lie far beyond science as such. This is not the place to launch into an analysis of the wider problems facing Europe; but two particular issues are in a real sense relevant, simply because they have been in our minds continuously during the planning of the colloquium and, rightly or wrongly, have played an important part in our motivation. It came as quite a shock to me, for example, to realize just how little communication there is between European earth scientists. The reasons for this are complex and transcend the earth sciences—history and language no doubt have much to do with it. It is also possible, of course, that this sense of isolation is a peculiarly British viewpoint. There is no doubt that for one reason or another the British geophysicist feels a far closer affinity towards his American than his European colleagues; and I suspect that there is a greater degree of communion between earth scientists in some of the continental European countries than there is between them and Britain.

The fact remains, however, that there is a much smaller sense of community between the geophysicists of Europe as a whole than there is, say, between the geophysicists of North America. It is perhaps significant that in organizing the colloquium the greatest problem we have had to face is not that of finding out what European geophysicists are doing but simply who and where they are. To be sure, most of the European geophysicists presumably publish their work; but much of it finds its way into national non-English language journals which are never translated. Indeed, it is much easier to discover what the geophysicists of the USSR are doing than what is happening in, say, France simply because the Soviet language barrier has been overcome by large-scale translation programmes. It is pertinent to note here too that the tremendous impact made in the west by Japanese geophysics is in large part due to the adoption of English as the prime language for scientific communication. It is not, of course, for an Englishman to sing the praises of the English language for fear of accusations of arrogance. Suffice it to say that written communication between European geophysicists is severely hampered by language differences and that in the short term a gathering of people offers the best chance for a much-needed exchange of information. The conference may be a primitive way of communicating but communication between European geophysicists is in a primitive state.

The second issue we have raised explicitly—and it is one



that I feel strongly about—is the altogether more subtle European barrier which has done, and continues to do, incalculable harm to science and earth science in Europe. I refer, of course, to the whole question of titular status and the hierarchy and, in particular, the position of the research student and the young lecturer or assistant professor. The really impressive thing about the AGU annual meeting is the way that both in and out of the formal programme the young and comparatively inexperienced mix with the older and more experienced on an equal personal footing to the benefit of both. The research student, for example, is not only supported financially to attend the meeting in the first place but is freely given precious time to present his results and ideas before as critical an audience of his discipline colleagues as he will probably ever find. And he will be judged purely on the merit of his work. This is not to say that the so-called eminent are not accorded the respect they merit but only that the younger scientist is made to feel a part of the geophysical community—and often, incidentally, a part of the decision-making process within science—in a way which many Europeans apparently find difficult to comprehend.

This whole matter reflects, of course, the ethos of American society in general which is in turn an accident of history. But it accounts in large part for the peculiar attraction of American scientific life for the younger scientist and is probably as important as the more obvious question of the availability of research funds. In any event we regard the colloquium in large part as a conscious attempt to emulate the AGU in this respect. Whether we have succeeded or not is, of course, another matter. It now looks as if the ratio of junior to senior scientists attending next week's colloquium will be about 1:2—a proportion which to me is disappointingly low and suggests that we have not been as successful as we would have liked. Indeed, it has been interesting to observe during the past few months the extent to which the system in Europe contains its own inbuilt constraints against change. If we have found one problem more intractable than that of identifying the established geophysicists in Europe it is that of contacting the many who work under them. We have been largely dependent for the moment on reaching the younger scientists through their titular superiors; but there is evidence to suggest that either the message has not always been passed on or, having been passed on, has not always received the necessary financial backing. However, it would be unrealistic to pretend that the habits and ways of thought established over generations can be changed overnight. But we have, I hope, made a modest start.

## The Programme

Within this philosophical framework we have tried to design a programme which covers the interests of European geophysicists, and which thus has a certain emphasis on Europe itself, but which at the same time involves the intellectual excitement inherent in the global approach to geophysics. The four mornings will be taken up by six thematic symposia (three of which will be running simultaneously at any given time), most of which try to strike a balance between invited papers of the review type and unsolicited presentations dealing with recent developments within the particular subject field. The first, for example, on the crustal and upper mantle structure of Europe and the Mediterranean will comprise a review of that subject by Dr S. Müller, a second review by Dr J. D. A. Zijdeveld on the palaeomagnetic evidence for crustal movement in Europe and fourteen submitted papers on subjects ranging from simulated data studies in crustal refraction seismology to the significance of ophiolite-chert sequences in the Middle East and Atlantic sea floor spreading. The second—the dynamics of geophysical fluids and plasmas—contains no invited reviews but a series of short presentations which vary from the general (convection with internal heat generation) to the particular (plasmopause whistlers observed in the UK).

The third symposium, coming, as it does, hard on the heels of the International Astronomical Union's symposium on the Moon being held at Newcastle upon Tyne, will include a higher proportion of general reviews. Thus the president of the AGU will speak on NASA's future programme of space exploration, Professor Harold Urey will outline the evidence for the origin of the Moon and the solar system, Dr R. Hide will talk about aspects of the major planets and Dr D. C. Tozer will expound on the thermal state of terrestrial planets. Going further down-scale there will also be reviews of the geology of the Moon, the mineralogy and chemistry of lunar rocks, the lunar magnetic field and Professor S. Tolansky's examinations of lunar glasses.

European space research is the subject of the fourth symposium and is of particular interest because it involves not only a session on remote sensing of the neutral atmosphere from space but a whole morning on opportunities for space research in Europe and details of ESRO's future programme. This will be held simultaneously with the theoretical geophysics symposium which is intended partly as a continuation of the highly successful series of symposia on geophysical theory and computers held in recent years under the auspices of the International Upper Mantle Committee. Within these two sessions Dr U. Schmucker will review theories of electromagnetic induction within the Earth, Dr R. L. Parker will give a general review of inversion theory and Dr D. B. Taylor will concern himself with the use of symbol manipulation in geophysical theory. Finally, the sixth symposium returns to the experimental scene with particular emphasis on the oxidation of basalts.

The three open sessions which take place simultaneously on three afternoons will comprise entirely submitted papers within the broad fields of planetary interiors, planetary atmospheres and interplanetary processes; and this is where the greatest contribution from the younger geophysicists is likely to come. The three frontiers lectures, on the other hand, are the preserve of the eminent. Thus Dr B. J. Mason, director general of the Meteorological Office, will speak on global circulation of the atmosphere including the large-scale effects of pollution, Professor G. Colombo of the University of Padua will lecture on evolutionary processes in the solar system and Professor K. J. Hsü of the Swiss Federal Institute of Technology will take advantage of Glomar Challenger's recent entry into the Mediterranean to discuss deep sea drilling (JOIDES) in the vicinity of Europe.

On the whole therefore the programme, like the corresponding AGU programme, is reasonably comprehensive. But there is one startling contrast which in a light-hearted way puts the thing into some sort of perspective. The fifty-second annual meeting of the AGU which takes place in Washington next month involves 102 sessions, 1,072 papers and between one and two thousand participants.

## The Future

In theory, and probably in practice too, the whole future of the annual European geophysical meeting should be decided in Reading next week. Part of one afternoon will be devoted to an open discussion of how successful the first meeting has been, whether the exercise is worth repeating and, if so, where the second meeting should be held. Although the first colloquium will be held in England, it was intended originally that subsequent ones should rotate around the capitals of Europe; but the success of such a course will obviously depend on whether any other country is willing to pick up the burden in subsequent years. However, there seems to be no reason why the colloquium should not go from strength to strength. I hope that ultimately the organizers of the many more specialized meetings that take place in Europe throughout the year will choose to time their gatherings to coincide with the colloquium. In that sense there is still a chance that we will end up with a European Geophysical Union.

# Polymorphism of Human Enzyme Proteins

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**Genetic variants of several enzymes have been found in human populations, and it seems that two unrelated individuals have very little chance of being identical in terms of protein structure.**

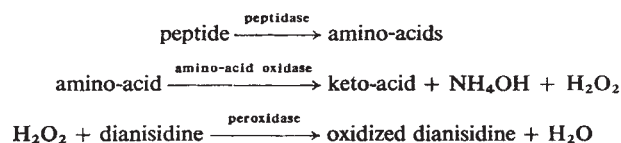
SINCE Landsteiner described the ABO blood group system<sup>1</sup>, it has become increasingly obvious that human individuality is demonstrable at the molecular level. It seems probable that almost every protein, structural or enzymatic, can be made to reveal some degree of variability in human populations. The application of starch gel electrophoresis<sup>2</sup> during the past decade has been especially fruitful in revealing molecular variability of particular enzymes in man. Using this method it has been possible to show common variants in approximately one enzyme in three in one of the major population groups<sup>3</sup>. This finding is the more remarkable in view of the fact that starch gel electrophoresis can reveal differences only in charge or size. If, as seems to be the case, the type of structural variation revealed by this technique results from the substitution of a particular amino-acid for another of a different charge, then because a charge difference will result from only about one in three of all possible amino-acid substitutions, it seems likely that many more differences in primary enzyme structure occur.

Enzymes which have been studied so far have been chosen principally because the reactions which they catalyse can be coupled to a suitable chromogenic reaction, so that the site of enzyme activity can be revealed as a sharp zone after electrophoresis. There is no reason to suppose, therefore, that selection on this basis would lead to the study of enzymes which are more commonly variable than those which are not amenable to this type of study. Some of the enzymes which have been studied are listed in Table 1.

The peptidases illustrate well the sort of information that can be obtained by the application of starch gel electrophoresis in association with a specific staining method. The occurrence of these enzymes in human tissues has been established for some time<sup>4</sup>; they catalyse the hydrolysis of the peptide bonds of small peptides consisting of two or three amino-acids. Their precise function is not known but they are probably involved in the terminal degradation of

proteins both in intestinal absorption and protein catabolism. Like most other enzymes which have been studied, peptidases have been obtained from red blood cells because blood samples are readily obtained from families—an essential prerequisite for genetic studies.

After starch gel electrophoresis at pH 7.5, peptidase activity can be detected by the following reaction sequence<sup>5</sup>,



This results in a brown precipitate at the site of activity. Using this method and various dipeptides and tripeptides, it has been possible to characterize six distinct peptidases in human red blood cells<sup>5,6</sup> (Table 2). Their relative electrophoretic mobilities are shown in Fig. 1. Although some of these enzymes have overlapping substrate specificities there are wide differences in their patterns of substrate specificity. Moreover, three of these enzymes apparently contain at least one reactive sulphhydryl group while the rest do not<sup>6</sup>. Further, gel filtration studies indicated that these enzymes also differ in molecular size<sup>7</sup>.

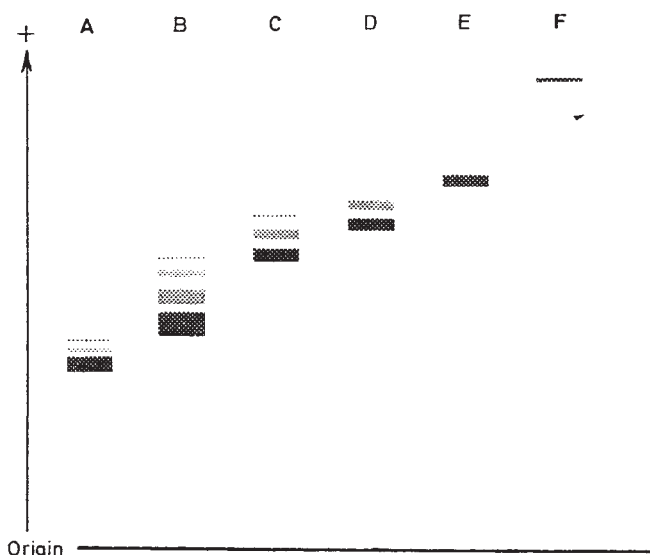
Biochemical evidence suggests that the six peptidases are distinct proteins, each with a characteristic primary structure and a characteristic specificity. It would be expected therefore that the primary structure of each peptidase would be coded for by a separate gene locus, so that a structural variant occurring in one peptidase would not be reflected in any of the others. This seems to be the case, and genetic studies have revealed further structural differences between these enzymes.

Peptidase A, a dipeptidase with a wide specificity (Table 2), has been studied in several different population groups. The variants shown in Fig. 2 are all rare except Pep A1, Pep A2-1 and Pep A2. Pep A1 is the commonest phenotype in

**Table 1** Frequencies of the Commonest Allele in Different Population Groups of Some of the Enzymes which have been Studied by Starch Gel Electrophoresis

|                                                | Europeans | Negroes   |
|------------------------------------------------|-----------|-----------|
| Red cell acid <sup>30</sup> phosphatase        | 0.6–0.7   | 0.8       |
| 6-Phosphogluconate <sup>31</sup> dehydrogenase | 0.96      | 0.72–0.87 |
| Phosphoglucosmutase                            |           |           |
| PGM <sub>1</sub> <sup>16</sup>                 | 0.76      | 0.76–0.79 |
| PGM <sub>2</sub> <sup>16</sup>                 | 1.0       | 0.95–0.99 |
| PGM <sub>3</sub> <sup>22</sup>                 | 0.74*     | 0.66*     |
| Peptidase A <sup>5,19</sup>                    | 1.0       | 0.70–0.90 |
| Peptidase D <sup>18</sup> (prolidase)          | 0.97      | 0.92      |
| Adenosine deaminase <sup>32</sup>              | 0.90      | 0.90      |
| Adenylate kinase <sup>33</sup>                 | 0.95      | 0.99      |

\* The commonest allele is not the same in the two populations studied for PGM<sub>3</sub>.



**Fig. 1** Relative electrophoretic mobilities of human red cell peptidases at pH 7.5.



**Table 2** Relative Activities of Human Red Blood Cell Peptidases against Various Amino-acid Peptides and Leucyl- $\beta$ -naphthylamide (Leu- $\beta$ -NA)

|                  | A   | B   | C   | D  | E  | F |
|------------------|-----|-----|-----|----|----|---|
| Val-Leu          | +++ | -   | -   | -  | -  | - |
| Gly-Leu          | +++ | -   | +   | -  | -  | - |
| Leu-Gly          | +++ | -   | +   | -  | -  | - |
| Gly-Phe          | ++  | -   | +   | -  | -  | - |
| Leu-Ala          | ++  | -   | ++  | -  | -  | - |
| Leu-Leu          | ++  | -   | ++  | -  | +  | - |
| Gly-Trp          | ++  | -   | ++  | -  | -  | - |
| Lys-Leu          | +   | +   | +++ | -  | +  | - |
| Lys-Tyr          | +   | +   | +++ | -  | +  | - |
| Pro-Phe          | ++  | +   | +++ | -  | -  | - |
| Pro-Leu          | ++  | +   | +++ | -  | -  | - |
| Phe-Leu          | ++  | ++  | +++ | -  | +  | - |
| Ala-Tyr          | ++  | +++ | +   | -  | ±  | - |
| Phe-Tyr          | ++  | ++  | +++ | -  | +  | - |
| Leu-Tyr          | +++ | ++  | +++ | -  | +  | - |
| Leu-Gly-Gly      | -   | +++ | -   | -  | ±  | - |
| Leu-Gly-Phe      | -   | +   | -   | -  | ±  | - |
| Leu-Leu-Leu      | -   | ++  | -   | -  | +  | + |
| Phe-Phe-Phe      | -   | ++  | -   | -  | ++ | ± |
| Tyr-Tyr-Tyr      | -   | +   | -   | -  | +  | + |
| Phe-Gly-Phe-Gly  | -   | -   | -   | -  | ++ | - |
| Leu- $\beta$ -NA | -   | -   | -   | -  | +  | - |
| Leu-Pro          | -   | -   | -   | ++ | -  | - |
| Phe-Pro          | -   | -   | -   | ++ | -  | - |

all the population groups studied so far, while Pep A2-1 and Pep A2 occur in appreciable frequencies only in African populations, or in populations of African origin such as the West Indian group. Table 3 shows that the frequencies of these variants in different populations are quite similar, although Pep A2-1 and Pep A2 seem to occur most often in the Babinga pigmies<sup>8</sup>, and are less common, as would be expected, in groups of mixed origin, such as the West Indians. A similar situation has been found with some other enzymes, for example glucose-6-phosphate dehydrogenase<sup>9</sup> and phosphoglucosmutase, locus *PGM<sub>2</sub>*<sup>10</sup>.

Family studies involving quite a large number of individuals indicate that the Pep A2-1 pattern occurs in people who are heterozygous for a pair of alleles which occur at an autosomal locus and which in homozygotes give rise to the Pep A1 and Pep A2 types. These alleles have been designated *Pep A<sup>1</sup>* and *Pep A<sup>2</sup>*. This type of inheritance is sometimes referred to as co-dominant because

both alleles are expressed in the heterozygote in contrast to the dominant-recessive inheritance in which only the dominant allele is apparently expressed in the heterozygote.

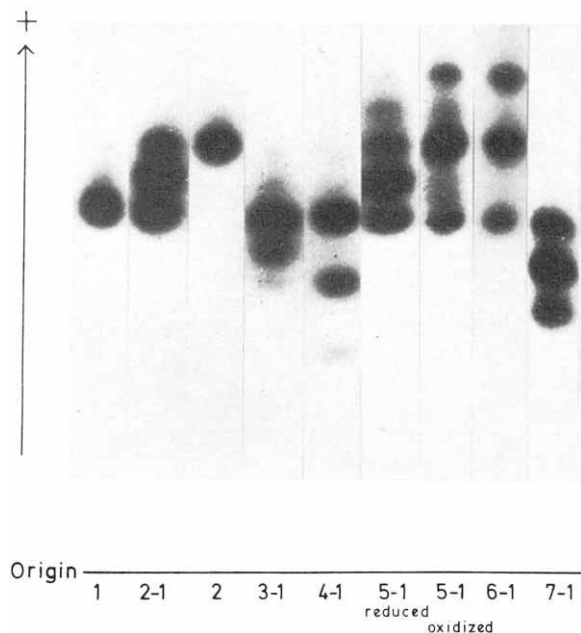
This type of study also makes possible inferences about the molecular structure of an enzyme. The electrophoretic pattern of the presumed heterozygote Pep A2-1 consists of three main zones of activity, a zone with a mobility equivalent to each of the presumed homozygotes, Pep A1 and Pep A2, and a band with almost exactly intermediate mobility, which is the most active. This type of electrophoretic pattern is thought to occur when the enzyme in homozygotes consists of at least two identical subunits<sup>11</sup>. If the subunit coded for by the *Pep A<sup>1</sup>* allele is represented by  $\alpha^1$  and that coded for by *Pep A<sup>2</sup>* is represented by  $\alpha^2$ , then if in the heterozygous individual both alleles are active and their corresponding subunits combine at random, there are three possible structures,  $\alpha^1\alpha^1$ ,  $\alpha^1\alpha^2$  and  $\alpha^2\alpha^2$ . Moreover, if the two subunits are synthesized at an equal rate and are equally active and stable, then the three zones would be expected to have activity approximately in the ratio of 1:2:1, and this seems to be the case.

**Table 3** Frequencies of Peptidase A Variants in Various Population Groups

|                  | Total | Peptidase A |     |   |     |     |     |     |     |
|------------------|-------|-------------|-----|---|-----|-----|-----|-----|-----|
|                  |       | 1           | 2-1 | 2 | 3-1 | 4-1 | 5-1 | 6-1 | 7-1 |
| Europeans        | 3,129 | 3,123       | —   | — | 1   | 3   | 1   | 1   | 1   |
| British resident |       |             |     |   |     |     |     |     |     |
| Negroes          | 636   | 554         | 78  | 3 | 1   | —   | —   | —   | —   |
| "Bantu"          |       |             |     |   |     |     |     |     |     |
| (South Africa)   | 100   | 87          | 11  | 2 | —   | —   | —   | —   | —   |
| Yoruba           |       |             |     |   |     |     |     |     |     |
| (Nigeria)        | 155   | 127         | 25  | 3 | —   | —   | —   | —   | —   |
| Cape Coloured    |       |             |     |   |     |     |     |     |     |
| (South Africa)   | 177   | 169         | 8   | — | —   | —   | —   | —   | —   |
| Cape Malay       |       |             |     |   |     |     |     |     |     |
| (South Africa)   | 104   | 96          | 8   | — | —   | —   | —   | —   | —   |
| Asiatic Indians  | 615   | 613         | —   | — | 1   | —   | 1   | —   | —   |

Another interesting feature of this particular enzyme is the occurrence of a series of rare phenotypes (Fig. 2). All these types are very rare, with frequencies of 1 in 1,000 or less in most population groups studied so far. Family studies suggest that these phenotypes occur in individuals who are heterozygous for one of a series of rare alleles, *Pep A<sup>3</sup>*, *Pep A<sup>4</sup>*, *Pep A<sup>5</sup>*, *Pep A<sup>6</sup>* and *Pep A<sup>7</sup>*, and the common allele *Pep A<sup>1</sup>*. In red blood cells two of these rare types, Pep A3-1 and Pep A4-1, have an electrophoretic pattern in which the characteristic electrophoretically slower components are weaker than the band corresponding to Pep A1. This might result if the subunits coded for by the rare alleles *Pep A<sup>3</sup>* and *Pep A<sup>4</sup>* were catalytically less active, were synthesized more slowly or were less stable. It seems likely that the last possibility is correct, for when these phenotypes are seen after electrophoresis of a tissue extract, for example placenta, the pattern is symmetrical and the three main bands have activity roughly in the ratio of 1:2:1. It seems likely that the stability difference is revealed in the red blood cell because of the relatively long half life of this type of cell, and presumably protein synthesis has ceased by the time that the nucleus is lost.

Another interesting variant in this group is Pep A5-1, which occurs in two forms; the pattern which is termed "reduced" in Fig. 2 is obtained when a fresh sample from an individual of this phenotype is electrophoresed, while the pattern termed "oxidized" is obtained if the sample is stored for a few days at 4° C before electrophoresis. If the stored sample is treated with mercaptoethanol, the resultant electrophoretic pattern is similar to that of the fresh sample. On the other hand, when a fresh sample

**Fig. 2** Electrophoretic patterns of peptidase A variants.

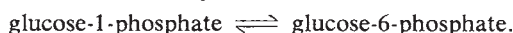
is treated with oxidized glutathione it has an electrophoretic pattern indistinguishable from that of the stored sample. It seems likely that the enzyme coded for by the variant allele, *Pep A<sup>5</sup>*, contains a reactive sulphhydryl group, which is not present in other peptidase A subunits, and which undergoes an exchange reaction with oxidized glutathione so that a half molecule of oxidized glutathione forms a disulphide bond with the enzyme<sup>12</sup>. The addition of the acidic peptide glutathione to the enzyme molecule would explain the increase in electrophoretic mobility after storage of haemolysates. This variant reacts with various reagents known to react with sulphhydryl groups and the electrophoretic changes produced by these reagents are consistent with the hypothesis I have outlined<sup>13</sup>.

It seems probable that the genetic code is common to most species<sup>14</sup>. If so, it is possible to postulate the particular amino-acid substitution that has occurred in the subunit coded for by the *Pep A<sup>5</sup>* allele. The only amino-acid which has a reactive sulphhydryl group which is found in proteins is cysteine. This amino-acid is neutral, and because there is an electrophoretic difference between the subunit coded for by *Pep A<sup>5</sup>* and that coded for by the common allele *Pep A<sup>1</sup>*, in that they migrate further towards the anode so that there has been an increase in net negative charge, cysteine must have been substituted for an amino-acid which would be positively charged at pH 7.5. This could be either lysine or arginine. Examination of the genetic code shows that while the substitution of cysteine for arginine could result from a change in single base pair, the substitution of cysteine for lysine could not. It seems possible therefore that in this variant a particular arginine residue has been replaced by cysteine. A similar hypothesis was proposed for *Pep A6-1*, suggesting that in this case glutamic acid is substituted for a particular lysine residue<sup>12</sup>.

A similar series of rare alleles has been described for several other enzymes, including peptidase B<sup>5,15</sup>, phosphoglucomutase<sup>16</sup>, and phosphohexose isomerase<sup>17</sup>.

A polymorphism of peptidase D (prolidase) has also been described<sup>18</sup>. Variants occur in all the major population groups so far studied. Recently variants have also been described of peptidase C in African pigmies<sup>19</sup>. In each case a variant of a particular peptidase is not associated with any change in electrophoretic pattern of any of the other peptidases. This finding supports the biochemical evidence that these enzymes are distinct proteins, and suggests that they are coded for by separate gene loci. Moreover, studies of two families indicate that the loci controlling the structures of these pairs of enzymes are not closely linked, but are either well separated on the same chromosome or situated on different chromosomes.

Another interesting group of isozymes which illustrates this type of variation in human populations is phosphoglucomutase (PGM). These enzymes are very specific phosphotransferases which catalyse the reaction



Using glucose-1-phosphate as the substrate it is possible to detect these isozymes after electrophoresis using glucose-6-phosphate dehydrogenase as a reagent, which oxidizes the glucose-6-phosphate, formed by the action of the phosphoglucomutase, with the concomitant reduction of NADP to NADPH. The reduced coenzyme reacts with phenazine methosulphate and a tetrazolium salt resulting in a deposit of insoluble formazan blue at the site of enzyme activity. This method, used after electrophoresis at pH 7.4, has revealed a polymorphism of phosphoglucomutase in human populations<sup>20</sup>. The electrophoretic patterns of the three phenotypes, PGM1, PGM2-1 and PGM2, are shown in Fig. 3. This polymorphism occurs in most populations, although gene frequencies differ.

Extensive family studies have shown that the different electrophoretic types result from the occurrence of a pair

of autosomal alleles. Individuals who are PGM1 or PGM2 are apparently homozygous for one of these alleles. The electrophoretic pattern (Fig. 3) consists of two bands, which in PGM2 migrate further towards the anode than those given by PGM1. In PGM2-1 the electrophoretic pattern consists of four bands, corresponding in mobility to each of the isozymes seen in the homozygotes. The family studies indicate that individuals who are PGM2-1 are heterozygotes, and the products of each allele seem to be synthesized in this phenotype.

Electrophoresis of red cell lysates also reveals a second series of isozymes migrating further towards the anode which do not show any variation associated with that which occurs in the slower moving group (PGM<sub>1</sub>). It was suggested that these faster moving isozymes (PGM<sub>2</sub>) were controlled by a second gene locus, and later some variants were described which supported this hypothesis<sup>10,16</sup>. Moreover, the available evidence suggests that the loci controlling the primary structures of PGM<sub>1</sub> and PGM<sub>2</sub> are not closely linked, but are either well separated on the same chromosome or on different chromosomes<sup>21</sup>.

Examination of placental extracts by electrophoresis revealed a third series of isozymes, PGM<sub>3</sub>, which are relatively less active and which migrate further towards the anode than either PGM<sub>1</sub> or PGM<sub>2</sub>. This group of isozymes of phosphoglucomutase also have common variation, which is genetically determined<sup>22</sup>, as studies of twin placentae have shown. This group of isozymes is apparently present in most tissues, although it has not been possible to demonstrate significant activity in red blood cells. Fibroblast cultures, however, have been used for the study of the segregation of the variants of PGM<sub>3</sub> in families, and the results agree well with those obtained from the study of twin placentae<sup>21</sup>.

The significance of the occurrence of these three different forms of phosphoglucomutase in man is not clear. It is interesting, however, that the electrophoretic patterns of the three groups of isozymes are quite similar, and that they seem to have similar if not identical molecular weights<sup>23,24</sup>. It is possible that they have related primary structures which have evolved by gene duplication from a single locus.

The implications of this type of variation in the primary structure of proteins in human populations are quite

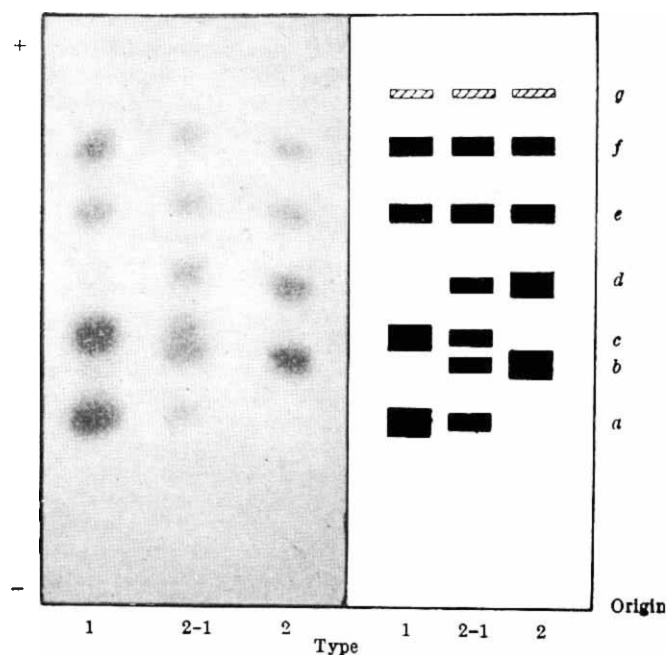


Fig. 3 Photograph and diagram of the electrophoretic patterns after electrophoresis of samples from individuals of the three common PGM<sub>1</sub> types. (From ref. 20.)

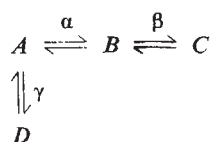


wide ranging, both for theoretical human biology and in the practical aspects of the study of the human organism. It is possible that common variants of a particular enzyme provide a pool of variability within the species, such that the constraints of the environment for the species as a whole are minimized, and the species is less vulnerable to environmental changes. Moreover, available techniques indicate that at least one in three enzymes has this type of variation. The chance of any two unrelated individuals being identical in terms of protein structure therefore seems to be extremely small. So far there is no experimental evidence to support the hypothesis that variation in the primary structure of enzyme proteins is the basis of much of the observed variation in the gross appearance of individuals. It seems reasonable to suppose, however, that many of the characters which are inherited in an obscure manner, and which are apparently controlled by many genes, are the result of structural variation at a molecular level of several different proteins.

The effect of a particular variant may well differ from one tissue to another. For example, a variant enzyme with a shorter half life than the usual form of the enzyme may not have any appreciable effect in a tissue with a fairly rapid cellular turnover, such as intestinal mucosa. On the other hand, in the erythrocyte, which has a relatively long half life and in which protein synthesis presumably ceases at about the time that the nucleus is lost, the activity of a particular structural form of an enzyme may be lost quite soon after the cell is released into the circulation. Such an unstable enzyme may result in a more rapid destruction of the erythrocyte, as for example occurs with some rare variants of enzymes in the anaerobic glycolytic pathway, such as pyruvate kinase<sup>25</sup>, or there may be no obvious effect until the individual is exposed to a toxic compound such as a drug or a particular type of food, as is seen with two different variants of glucose-6-phosphate dehydrogenase<sup>26,27</sup>. In one case, treatment with the antimalarial drug, primaquine, induces a haemolytic crisis and, in the other, ingestion of the fava bean has a similar effect.

Another consequence of the occurrence of structural variants of enzymes would be that heterozygotes would have at least two structural forms of the enzyme, and if the enzyme were a dimer there would be three isozymes, four for a trimer and five for a tetramer. The proportions in which these isozymes were present in any tissue would clearly depend on several factors, including the stability of the subunits and of different combinations of subunits. The apparent activity would also depend on the relative catalytic efficiency of particular combinations of subunits. It also seems possible that these isozymes are present in different proportions throughout the life of the cell. Whether or not this phenomenon has any significance for the whole organism is not known.

Another possible effect of a difference in half life, and therefore of activity, of an enzyme may occur when the enzyme affected is part of a metabolic scheme which is made up of different pathways. If we consider a hypothetical three-enzyme system such as that illustrated, and if in the normal situation with normal concentrations of  $\alpha$ , and  $\beta$ , 25 per cent of  $A$  is converted to  $D$ , then if  $\alpha$  is replaced by  $\alpha'$  which is less stable or catalytically less active, the rate of conversion of  $A$  to  $B$  would be reduced and more of  $A$  could be metabolized to  $D$ .



Such a situation in itself might not, in most conditions, have any demonstrable effect on the individual. But if the

individual is faced with a metabolic challenge in which the concentrations of  $B$  or  $C$  are critical, then the effect may well be quite dramatic, as in the case of primaquine sensitivity<sup>26,27</sup>.

On the other hand, the accumulation of a high concentration of  $D$  may in itself be toxic, and this situation is found with some inborn errors of metabolism, such as galactosaemia<sup>28,29</sup>, although in these cases the level of activity of the affected enzyme is usually very low indeed, and perhaps beyond the limits of detection. There are of course many possible variations within even the simple scheme I have outlined. It seems probable that these "enzymopathies" are part of the same general phenomenon as the rare variants, but in this case the affected individual can apparently synthesize only an unstable enzyme, as an inactive enzyme is perhaps completely incapable of synthesizing the particular enzyme protein.

If the enzyme concerned in a particular metabolic error is common to most tissues, the study of the enzyme in erythrocytes makes it possible to study the enzyme without recourse to biopsy procedures. The occurrence of a polymorphism can provide good evidence that the enzymes under investigation in different tissues are the products of the same gene locus and are therefore probably structurally identical.

<sup>1</sup> Landsteiner, K., *Wein. Klin. Wschr.*, **14**, 132 (1901).

<sup>2</sup> Smithies, O., *Biochem. J.*, **61**, 629 (1955).

<sup>3</sup> Harris, H., *Proc. Roy. Soc., B*, **174**, 1 (1969).

<sup>4</sup> Adams, E., McFadden, M., and Smith, E. L., *J. Biol. Chem.*, **198**, 663 (1952).

<sup>5</sup> Lewis, W. H. P., and Harris, H., *Nature*, **215**, 351 (1967).

<sup>6</sup> Lewis, W. H. P., *Symp. Zool. Soc.*, **26**, 93 (1970).

<sup>7</sup> Lewis, W. H. P., and Harris, H., *Ann. Human Genet.*, **33**, 89 (1969).

<sup>8</sup> Santachiara-Benerecetti, S. A., *Atti. Assoc. Genet. Ital.*, **14**, 145 (1969).

<sup>9</sup> Boyer, S. H., Porter, I. H., and Weilbacher, R. G., *Proc. US Nat. Acad. Sci.*, **48**, 1868 (1962).

<sup>10</sup> Hopkinson, D. A., and Harris, H., *Nature*, **208**, 410 (1965).

<sup>11</sup> Shaw, C. R., *Science*, **149**, 936 (1965).

<sup>12</sup> Lewis, W. H. P., Corney, G., and Harris, H., *Ann. Human Genet.*, **32**, 35 (1968).

<sup>13</sup> Hopkinson, D. A., and Sinha, K. P., *Ann. Human Genet.*, **33**, 139 (1969).

<sup>14</sup> Crick, F. H. C., *Proc. Roy. Soc., B*, **167**, 331 (1967).

<sup>15</sup> Blake, N. M., Kirk, R. L., Lewis, W. H. P., and Harris, H., *Ann. Human Genet.*, **33**, 301 (1969).

<sup>16</sup> Hopkinson, D. A., and Harris, H., *Ann. Human Genet.*, **30**, 167 (1966).

<sup>17</sup> Dettler, J. C., Ways, P. O., Giblett, E. R., Baughan, M. A., Hopkinson, D. A., Povey, S., and Harris, H., *Ann. Human Genet.*, **31**, 329 (1968).

<sup>18</sup> Lewis, W. H. P., and Harris, H., *Ann. Human Genet.*, **32**, 317 (1969).

<sup>19</sup> Sanachiara-Benerecetti, S. A., *Amer. J. Human Genet.*, **22**, 232 (1970).

<sup>20</sup> Spencer, N., Hopkinson, D. A., and Harris, H., *Nature*, **204**, 742 (1964).

<sup>21</sup> Parrington, J. M., Cruikshank, G., Hopkinson, D. A., Robson, E. B., and Harris, H., *Ann. Human Genet.*, **32**, 27 (1968).

<sup>22</sup> Hopkinson, D. A., and Harris, H., *Ann. Human Genet.*, **31**, 395 (1968).

<sup>23</sup> McAlpine, P. J., Hopkinson, D. A., and Harris, H., *Ann. Human Genet.*, **34**, 177 (1970).

<sup>24</sup> Monn, E., *Intern. J. Protein Res.*, **1**, 73 (1969).

<sup>25</sup> Valentine, W. N., Tanaka, K. R., and Miwa, S., *Trans. Assoc. Amer. Physicians*, **74**, 100 (1961).

<sup>26</sup> Carson, P. E., Flanagan, C. L., Ickes, C. E., and Alving, A. S., *Science*, **124**, 484 (1956).

<sup>27</sup> Szeinberg, A., Sheba, C., Hirshom, N., and Bodongi, E., *Blood*, **12**, 603 (1957).

<sup>28</sup> Harris, H., *Human Biochemical Genetics* (Cambridge University Press, 1959).

<sup>29</sup> Crome, L., and Stern, J., *The Pathology of Mental Defect* (Churchill, London, 1967).

<sup>30</sup> Hopkinson, D. A., Spencer, N., and Harris, H., *Nature*, **199**, 969 (1963).

<sup>31</sup> Giblett, E. R., *Genetic Markers in Human Blood*, 491 (Blackwell, Oxford, 1969).

<sup>32</sup> Spencer, N., Hopkinson, D. A., and Harris, H., *Ann. Human Genet.*, **32**, 9 (1968).

<sup>33</sup> Fildes, R. A., and Harris, H., *Nature*, **209**, 261 (1966).

# Super-heavy Elements in Extraterrestrial Samples

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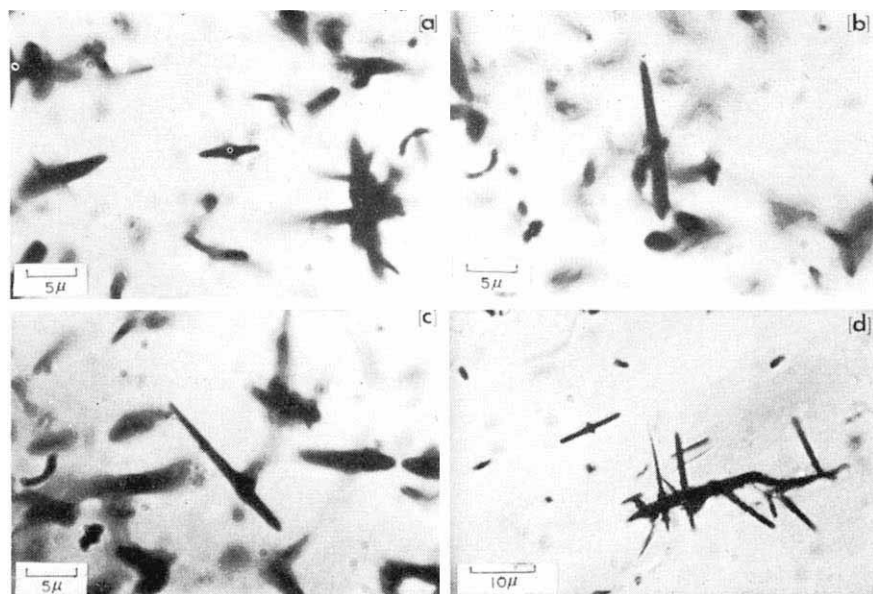
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**Fossil track studies in several meteorites and in lunar dust show that super-heavy ( $Z > 110$ ) transuranic elements were indeed present when these objects solidified.**

THE prediction<sup>1,2</sup> of an island of stability at mass  $\sim 284$  and charge  $\sim 110$  has prompted a search for its stored signatures/records in meteorites<sup>3-5</sup> and terrestrial minerals<sup>6-8</sup>. We report here observations which provide conclusive proof for the presence at some time of super-heavy transuranic elements in meteorites (and lunar dust<sup>9</sup>). The search for super-heavy elements in the meteorites is based on analyses of (1) the isotopic composition of krypton and xenon<sup>3,4</sup> and (2) the lengths of fossil tracks<sup>5,10</sup>. The former method is based on analyses of the whole sample but in the latter only silicate crystals, where fission records can be studied<sup>11,12</sup>, are examined. The rare gas studies can be made in carbonaceous chondrites type 1, the least differentiated objects in which fossil track examinations are not possible, and the method is thus restricted to the study of heavily differentiated meteorites in which the uranium and heavy elements are also largely rejected from the crystal lattice to the grain boundaries. We have, however,

preferred to use the fossil track method in spite of its inability to determine the primordial concentrations, because the method is direct and unambiguous. Our aim here has therefore been primarily to determine whether super-heavy transuranic elements are present in ancient objects of the solar system.

The fossil track length technique for checking on the presence of super-heavy elements has been tried<sup>5</sup>, but yielded a negative result, partly because of an indirect approach. Our method, however, differs in two ways from this and has not been attempted previously except in a preliminary study of Apollo 11 "fines"<sup>13</sup>. We studied first the lengths of tracks completely confined within the mineral grain, that is we obtained the total recordable length of fission fragments, in contrast to the method adopted earlier<sup>5</sup>, where the partial lengths of tracks on a fractured crystal surface were studied; interpretation of the observed length distribution data so obtained becomes a problem because of contributions from cosmic ray nuclei<sup>14</sup>. The "complete" confined tracks (due to spontaneous fission as well as cosmic ray heavy nuclei) are very easily observed after over-etching by factors of 2-5 X and are termed TINTS or TINCLES depending on whether the etching channel or surface across which tracks develop is provided by a surface track (a track-in-track event: TINT, for details see Lal *et al.*<sup>15</sup>), or a canal/fissure/cleavage in the crystal (a track in the cleavage: TINCLE). Figs. 1-3 show examples of TINTS and TINCLES. MacDougall *et al.* are preparing an article reporting the development of TINT/TINCLE tracks, in which they will show



**Fig. 1** Photomicrographs of "complete" tracks (TINT type), etched via the canal of a surface track (only partially visible in the photograph) in meteoritic silicates. The TINTS are in the centre in *a*, *b* and *c*. In *d* one TINT is north-west of the TINCLE field. Crystals are Moore County feldspars (*a*, *b*, *c*) and pyroxene (*d*). Recorded track lengths correspond to V, Cr, Mn and Fe.



that if completely developed track holes are plated with silver to increase optical contrast, the lengths can be measured accurately and that, after re-etching, tracks of  $< 30 \mu\text{m}$  do not increase further by more than  $0.5\text{--}1.0 \mu\text{m}$ .

We have used a simple method for studying a fission track-rich sample in extraterrestrial samples where most tracks are usually due to cosmic ray nuclei. This technique depended on our observation that in the case of TINCLES, the number of tracks in a cleavage is sometimes too large compared with that in others. Theoretically, if the surface track density is  $\rho$  (tracks  $\text{cm}^{-2}$ ), then for an open crevasse or a slit permitting free etching of tracks, the number of tracks revealed per  $100 \mu\text{m}$  length along the slit would be expected to be approximately  $10^{-6} \rho G d$ , the cleavage number  $N_{cl}$ , where  $d$ , in  $\mu\text{m}$ , is the depth of focus for observing TINCLES along a cleavage plane and  $G$  is a geometrical factor for revealing tracks of different length, which depends on the cleavage gap and is close to 1.0 (because we are interested in tracks which are long compared with the cleavage gap,  $< 1 \mu\text{m}$  (Figs. 1–3)). Usually  $d$  is about  $1 \mu\text{m}$  and for  $\rho = 5 \times 10^6$ , we would expect to see 5 tracks per  $100 \mu\text{m}$  length. The observed values of  $N_{cl}$  are theoretically the same in most cases but occasionally, and in practically all the meteorite samples studied, we observed very large  $N_{cl}$  values, exceeding the expected values by factors of up to 100. In such cases, excess tracks are clearly due to rejection of uranium, plutonium and other elements by the crystal along exsolution/flow planes which we will call “cleavage” planes from now on only for convenience. In a way, such regions can be considered as grain boundaries within the crystals. We obtained experimental proof for this by studying neutron induced tracks in meteoritic and lunar crystals.

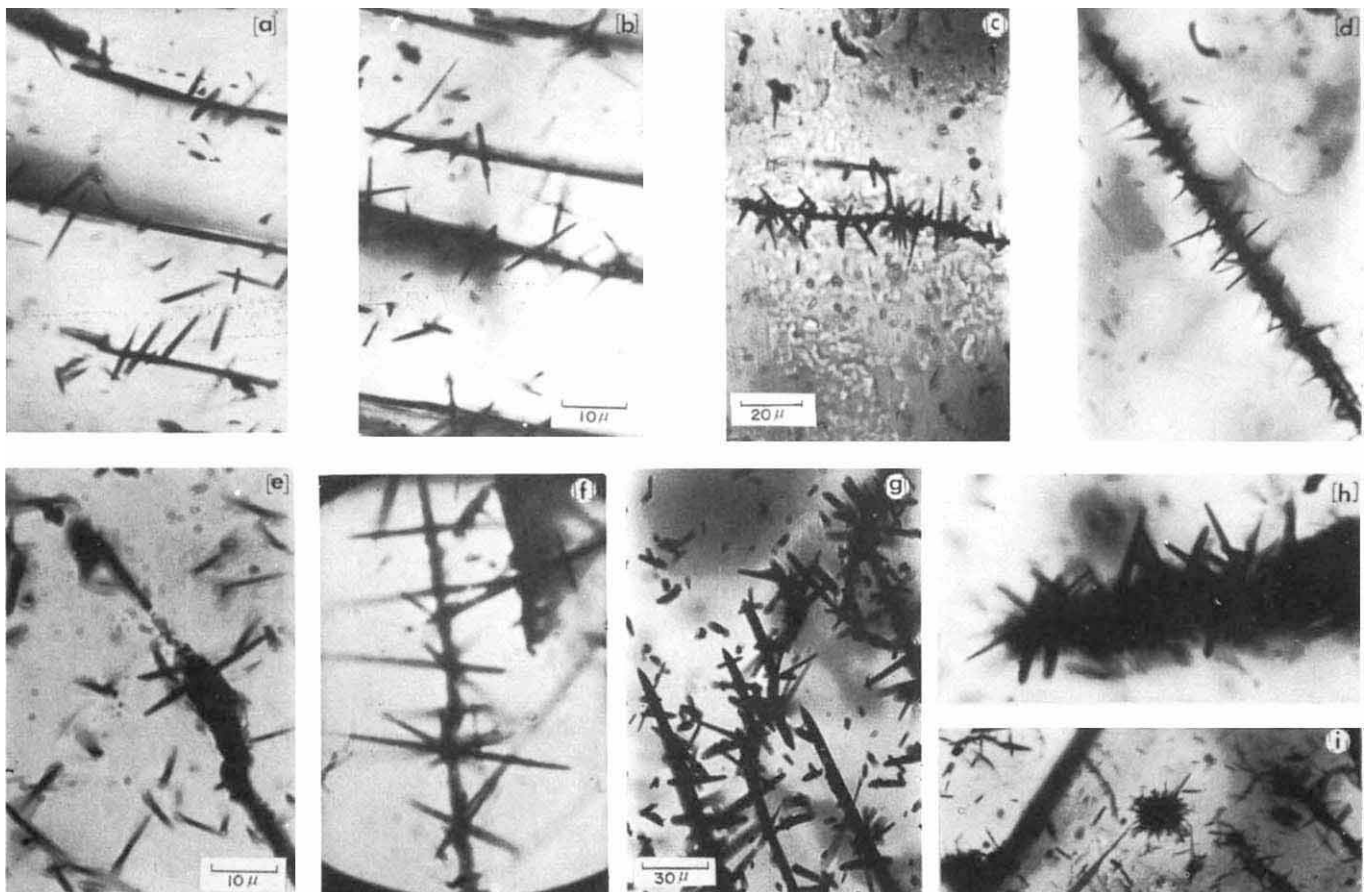
Thus, by a combination of our two innovations we have been able to study a fission-rich sample of tracks for their “total” recordable lengths. The data provide conclusive proof

that super-heavy elements existed in the early history of the solar system.

## Experimental Data and Discussion

The techniques for mounting, grinding, polishing, etching and viewing tracks with a high contrast (by plating track holes with silver) have been described<sup>9,14</sup>. The measured total recordable lengths are plotted in Fig. 4 for three meteorites and also Apollo 11 and 12 rocks and dust<sup>9</sup>. The data refer to different pyroxenes (see caption of Fig. 4) only; feldspar data are not included because the residual and recorded ranges differ.

The theoretically calculated total ranges of fission fragments from  $^{235}\text{U}$ ,  $^{238}\text{U}$ ,  $^{244}\text{Pu}$  and super-heavy fission tracks (both fragments) are given in Table 1, for pyroxenes of different composition. From direct measurements of n-induced  $^{235}\text{U}$  fission fragment tracks in pyroxene crystals from Angra dos Reis, Moore County and Kapoeta, the recordable track lengths were experimentally measured to lie between  $14.3\text{--}15.5 \mu\text{m}$ , with a standard deviation of about  $1.0 \mu\text{m}$  in each case (results due to Bhandari *et al.*, unpublished work). Identical length distribution with a peak at  $14.3 \mu\text{m}$  has been seen for the fossil tracks from Angra dos Reis, where cosmic ray nuclei tracks are present at ultra low densities,  $\ll 10^4 \text{ cm}^{-2}$ . Similarly, in other meteorites (Kapoeta, Moore County and Norton County), the fossil tracks in fission-rich zones, for example in Sun-burst events (Fig. 2) and in TINCLES of high  $N_{cl}$  track lengths, have a peak around  $15 \mu\text{m}$  but the distribution extends farther to longer tracks. Therefore, the measured lengths of both fossil and induced tracks in  $^{238}\text{U}$  ( $^{244}\text{Pu}$ ) and  $^{235}\text{U}$  are smaller by about  $3\text{--}4 \mu\text{m}$  than the theoretical ranges, indicating that about  $2 \mu\text{m}$  is not recorded for each fragment at the end of its range where the primary ionization of the fragment drops below



**Fig. 2** Photomicrographs of TINCLES in meteorites, Norton County (*a, b*, pyroxene), Crab Orchard (*c, d*, feldspar) and Moore County (*e, f, g*, feldspar). Examples of TINCLE fields of normal and higher “cleavage numbers”,  $N_{cl}$ , within a given sample can be seen. *h* and *i* respectively show super-high  $N_{cl}$  TINCLE field and a “Sun-Burst” (or “Sun-Flower”) event due to fissions at the inclusion; in both these cases length measurements are not possible.

the critical value above which only tracks are etchable<sup>19</sup>. The recorded lengths for fission fragment tracks due to the fission of super-heavy elements would be expected to be similarly shorter, by  $< 3 \mu\text{m}$ , than the predicted ranges, corresponding to mean recorded lengths of 17–24 microns (see Table 2). This is based on theoretical calculations essentially following the treatment of Price *et al.*<sup>20</sup>.

In the meteorite Angra dos Reis, the cosmic ray tracks are responsible for fewer than 1 track in  $10^3$ . The TINCLE fields in Angra dos Reis have very large  $N_{cl}$  values (100–500, Fig. 3) but it usually becomes difficult to measure tracks when  $N_{cl} > 100$ . Where possible, we looked for long tracks, and found seven longer than  $18 \mu\text{m}$ , the upper limit for neutron induced tracks, in a total of  $\sim 1,500$  TINCLES; two of these are very long indeed:  $21.6$  and  $25.8 \mu\text{m}$ . There is no correction for cosmic ray (CR) tracks in Angra dos Reis samples, unlike in the other meteorites, because  $\rho_{CR}$  is estimated by Bhandari *et al.* to be less than  $10^4 \text{ cm}^{-2}$ , and more than 80% of the cosmic ray tracks are short ( $< 13 \mu\text{m}$ ) due to Fe, Mn, Cr and V nuclei (Table 2). The long tracks in Angra dos Reis therefore constitute a unique proof for higher energy release fissions compared with  $^{244}\text{Pu}$  and  $^{238}\text{U}$ .

For other samples (data presented in Fig. 4 and Table 2), corrections for cosmic ray tracks are not large and can be made easily. Based on empirical observations in meteorites and lunar samples<sup>9,14</sup>, where  $\rho(\text{CR}) \gg \rho(\text{fission})$ , the upper limits of the relative contributions of tracks due to cosmic ray nuclei in different length intervals are given in Table 2. Fortunately, because iron is most abundant among the 22–30 charge elements, fragmentations of these nuclei only alter the correction factors for tracks of  $< 13 \mu\text{m}$  which are due solely to cosmic ray nuclei.

Data in Fig. 2 and Table 2 show that for a given specimen, when the length distribution for TINCLES of high  $N_{cl}$  is examined, excess tracks increase in both the 13–16 and the 16–22  $\mu\text{m}$  interval, relative to iron nuclei. (The distribution at  $15 \mu\text{m}$  has a prominent peak but that for  $> 16 \mu\text{m}$  seems to be diffuse.) Thus, consistently for all samples (except Apollo rocks) we see fission contributions both in the  $^{244}\text{Pu}$ ,  $^{238}\text{U}$  region as well as in that expected for super-heavy elements (see footnote to Table 2). Could these tracks have a non-spontaneous fission origin? Fleischer *et al.*<sup>11,12</sup> have considered the possible origins of fossil tracks but their work minimizes the probability of other sources whereas, in our work, many of the possibilities (however remote) such as neutron-induced fission of heavy elements present in pyroxenes, monopoles (?), can be dismissed immediately because our study

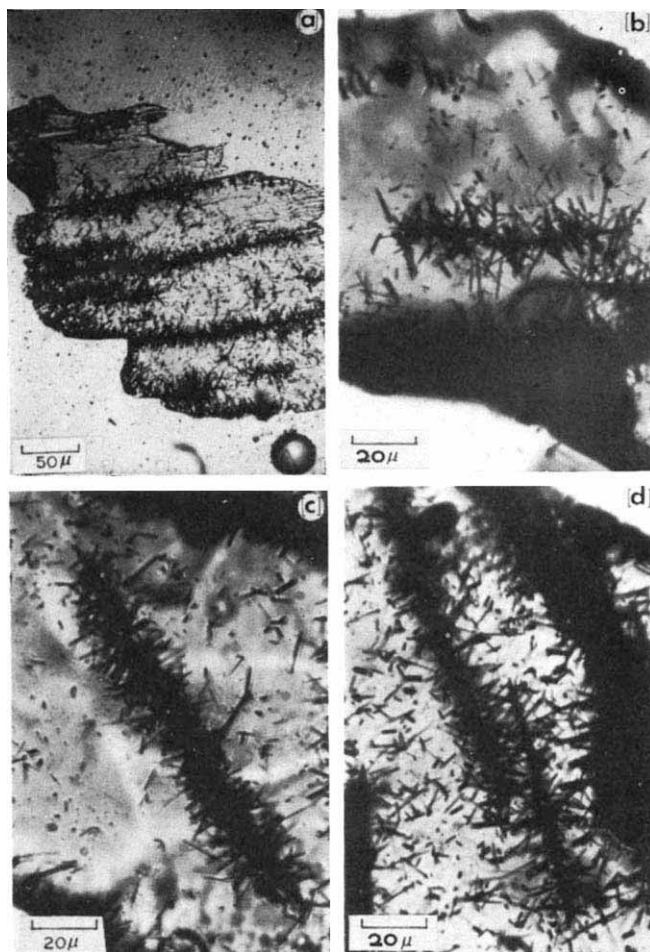


Fig. 3 TINCLE fields in Angra dos Reis. Typical  $N_{cl}$  values are 100 (a, b), an order of magnitude larger than expected. Even higher  $N_{cl}$  TINCLE fields are seen (a, c and d).

is based on a direct experimental comparison on length distribution between meteoritic and lunar specimens exposed to a varying dose of cosmic rays. For example, excess fission tracks can be seen in Apollo "fines", but not in rocks which are young ( $^{244}\text{Pu}$  had certainly become extinct in these rocks when they solidified). In Table 2 we have comparative data for different meteorite specimens of Norton County, with

Table 1 Calculated \* Total Ranges of Fission Fragments (in  $\mu\text{m}$ ) for Various Minerals

| Mineral (specific gravity)<br>chemical composition                                                                                                                          | $^{235}\text{U}$<br>induced | $^{238}\text{U}$<br>spontaneous | $^{244}\text{Pu}$<br>spontaneous | Super-heavy †   |                  |                 |                  |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------|---------------------------------|----------------------------------|-----------------|------------------|-----------------|------------------|
|                                                                                                                                                                             |                             |                                 |                                  | Z = 114<br>Sym. | Z = 114<br>Asym. | Z = 126<br>Sym. | Z = 126<br>Asym. |
| Pigeonite (3.38)<br>$\text{Mg}_{0.9} \text{Fe}_{0.96} \text{Ca}_{0.08} (\text{Si}_2\text{O}_6)$                                                                             | 18.0                        | 18.2                            | 18.3                             | 20.9            | 23.6             | 23.3            | 25.2             |
| Augite (3.4)<br>$\text{Ca}_{0.81} \text{Mg}_{0.75} \text{Fe}_{0.37} \text{Na}_{0.06} \text{Al}_{0.34} (\text{Si}_{1.66}\text{O}_6)$                                         | 17.5                        | 17.7                            | 17.8                             | 20.2            | 22.9             | 22.6            | 24.5             |
| Angrite (3.25)<br>$\text{Ca}_{0.5} \text{Mg}_{0.27} \text{Fe}_{0.15} \text{Al}_{0.04} \text{Ti}_{0.03}$<br>$\text{Na}_{0.01} (\text{Si}_{0.87} \text{Al}_{0.13})\text{O}_3$ | 18.4                        | 18.6                            | 18.7                             | 21.3            | 24.1             | 23.7            | 25.7             |
| Diopside (3.27)<br>$\text{Ca Mg} (\text{Si}_2\text{O}_6)$                                                                                                                   | 17.7                        | 17.9                            | 18.0                             | 20.5            | 23.2             | 22.9            | 24.8             |
| Enstatite (3.1)<br>$(\text{Mg}_{0.94} \text{Fe}_{0.06}) \text{SiO}_3$                                                                                                       | 18.2                        | 18.4                            | 18.5                             | 21.1            | 23.9             | 23.5            | 25.5             |
| Bronzite (3.3)<br>$(\text{Mg}_{0.79} \text{Fe}_{0.21}) \text{SiO}_3$                                                                                                        | 17.6                        | 17.8                            | 17.9                             | 20.4            | 23.1             | 22.7            | 24.6             |
| Hypersthene (3.65)<br>$\text{Mg}_{0.6} \text{Fe}_{0.4} (\text{SiO}_3)$                                                                                                      | 16.5                        | 16.7                            | 16.7                             | 19.1            | 21.6             | 21.2            | 23.0             |

\* Based on Range-Energy Computer Code (RANGENER) of Henke and Benton (1967)<sup>16</sup>. Fission energy release values are based on references 2, 17 and 18 ( $^{235}\text{U}$ —induced, 167 MeV;  $^{238}\text{U}$ —spontaneous, 170 MeV;  $^{244}\text{Pu}$ —spontaneous, 172 MeV;  $^{114}\text{Sh}^{298}$ —spontaneous, 235 MeV;  $^{126}\text{Sh}^{310}$ —spontaneous, 293 MeV). Sh = Super-heavy.

† Calculations for the symmetric and asymmetric fission modes are analogous to those discussed by Rao<sup>22</sup>.



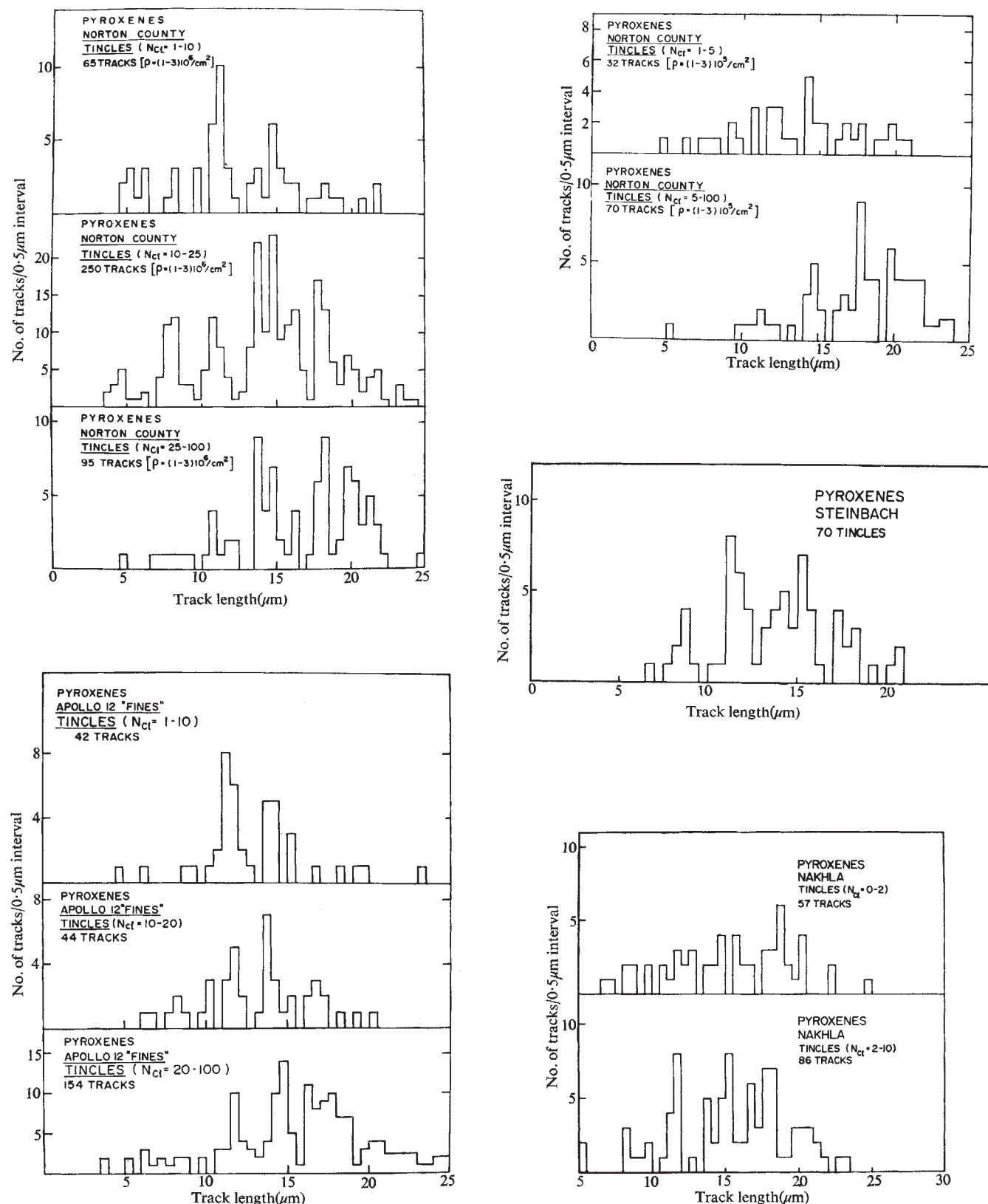


Fig. 4 Observed length distribution of "complete" tracks in three meteorites and in lunar fines returned on Apollo 12 mission, for both TINTS and TINCLES ( $N_{cl}$ , 1-100). All data refer to pyroxenes; enstatite in Norton County, augite in Apollo fines, bronzite in Steinbach and diopside in Nakhla. With increase in  $N_{cl}$  there occurs an increase in 13-16 μm tracks relative to the number of tracks due to cosmic ray iron (10-13 μm) and lower charge nuclei (Cr and Mn, 4-10 μm). In Nakhla, the lowest observed  $N_{cl}$  values are higher than expected because of the high uranium content of diopside.

$\rho = 1.7 \times 10^6$ , and for different  $N_{cl}$  values for a given meteorite specimen. Any other source of tracks could not have led to the systematic pattern visible, namely that in a sample of tracks rich in fission, the Pu+U and super-heavy tracks increase relative to iron. The ratios of the tracks contributed by Fe, U+Pu and super-heavy are given in Table 2. The recorded length for  $^{244}\text{Pu}$  fission is close to that for  $^{238}\text{U}$  (spontaneous) or n-induced  $^{235}\text{U}$  fission (Table 1) and therefore  $^{244}\text{Pu}$  events are not distinguishable by length measurements alone. We have therefore calculated expected  $^{238}\text{U}$  fission tracks  $\text{cm}^{-2}$  (in  $4.5 \times 10^9$  yr) on the basis of the experimentally determined  $^{238}\text{U}$  concentration in the pyroxenes studied using the neutron activation method<sup>12</sup>. These results and the estimated  $\rho(^{244}\text{Pu})/\rho(^{238}\text{U})$  ratios are given in Table 3. There is a remarkable internal agreement within the different samples, except for Kapoeta. The  $\rho(^{244}\text{Pu})/\rho(^{238}\text{U})$  ratios range from 19 to 38 for Angra dos Reis, Moore County and Apollo "fines". Earlier measurements<sup>10,21</sup> gave values of 50–80 for diopside in Toluca and El Taco.

Thus, in all the meteorites studied and in Apollo 11 and 12 "fines",  $^{244}\text{Pu}$  contributions to all fission tracks in the 13–16  $\mu\text{m}$  interval (in pyroxenes) are very similar. The range of corre-

sponding variations for super-heavy to (U + Pu) is more variable and may reflect a higher chemical sensitivity of super-heavy element to the mineral. An accurate average value for the relative contributions in the samples studied for tracks due to U, Pu, super-heavy fissions is 0.03, 1, 1, respectively. We would like to repeat that these values do not refer to primordial concentrations because of chemical differentiation. Also the  $\rho(^{244}\text{Pu})/\rho(\text{super-heavy})$  ratio is of the order of 200 in Angra dos Reis.

We believe that the experimental results presented here provide conclusive proof that super-heavy transuranic elements exist in lunar fines. The total number of tracks studied for the measurement of complete lengths in the present work, including those in lunar samples<sup>9</sup>, is 1,600 out of which 933 are longer than 13  $\mu\text{m}$ , out of which excess tracks, that is those due to fission of  $^{244}\text{Pu}$  (and  $^{238}\text{U}$ ) and super-heavy elements, number 356 and 338 respectively (Table 2). Thus, interpretations are not subject to uncertainties due to statistics. We do not, however, know the charge(s) of the super-heavy element(s), but the measured recordable fission ranges are quite consistent with those expected for elements with atomic number 114.

We thank Drs G. Arrhenius, R. R. Daniel, M. G. K. Menon

**Table 2** Relative Fission Contributions to Tracks in Meteorite and Lunar Samples

| Sample              | Track type measured | $N_{cl}$ | Fossil track density $\rho(\text{cm}^{-2})$ | No. of tracks measured |                                    | Total in 13–16 $\mu\text{m}$ interval | Relative track abundances (Fe (10–13 $\mu\text{m}$ ) = 100 tracks)     |                                           | Estimated * fission tracks from super-heavy nuclei | Relative contributions to tracks |                    |
|---------------------|---------------------|----------|---------------------------------------------|------------------------|------------------------------------|---------------------------------------|------------------------------------------------------------------------|-------------------------------------------|----------------------------------------------------|----------------------------------|--------------------|
|                     |                     |          |                                             | Total                  | Due to iron (10–13 $\mu\text{m}$ ) |                                       | Estimated * due to (U + Pu) fission (that is, corrected for CR tracks) | Total in the 16–22 $\mu\text{m}$ interval |                                                    | (U + Pu)† Fe                     | Super-heavy U + Pu |
| Moore County        | TINTS               | —        | $3 \times 10^6$                             | 144                    | 29                                 | 210                                   | 185                                                                    | 101                                       | 83                                                 | 1.8                              | 0.45               |
| Norton County       | TINTS               | —        | $1.7 \times 10^6$                           | 162                    | 50                                 | 126                                   | 101                                                                    | 54                                        | 36                                                 | 1                                | 0.36               |
| Norton County       | TINCLES             | 1–10     | $1.7 \times 10^6$                           | 65                     | 23                                 | 83                                    | 58                                                                     | 40                                        | 22                                                 | 0.58                             | 0.38               |
| Norton County       | TINCLES             | 10–25    | $1.7 \times 10^6$                           | 250                    | 36                                 | 280                                   | 255                                                                    | 178                                       | 160                                                | 2.5                              | 0.63               |
| Norton County       | TINCLES             | 25–100   | $1.7 \times 10^6$                           | 95                     | 11                                 | 245                                   | 220                                                                    | 400                                       | 382                                                | 2.2                              | 1.7                |
| Norton County       | TINCLES             | 1–5      | $1.7 \times 10^5$                           | 40                     | 13                                 | 77                                    | 52                                                                     | 92                                        | 74                                                 | 0.52                             | 1.4                |
| Norton County       | TINCLES             | 5–100    | $1.7 \times 10^5$                           | 70                     | 7                                  | 157                                   | 132                                                                    | 730                                       | 712                                                | 1.32                             | 5.5                |
| Steinbach           | TINCLES             | 1–20     | $1.3 \times 10^6$                           | 70                     | 21                                 | 128                                   | 103                                                                    | 61                                        | 43                                                 | 1.0                              | 0.42               |
| Nakhla              | TINCLES             | 1–10     | $4.25 \times 10^5$                          | 143                    | 25                                 | 184                                   | 159                                                                    | 220                                       | 202                                                | 1.6                              | 1.3                |
| Apollo 11, 12 rocks | TINTS and TINCLES   | 1–5      | $7 \times 10^6$                             | 323                    | 123                                | 37                                    | 12                                                                     | 2                                         | ~0                                                 | 0.12                             | ~0                 |
| Apollo 11, 12 fines | TINCLES             | 1–10     | $\sim 7 \times 10^6$                        | 42                     | 22                                 | 59                                    | 34                                                                     | 23                                        | 5                                                  | 0.34                             | 0.15               |
| Apollo 11, 12 fines | TINCLES             | 10–20    | $\sim 7 \times 10^6$                        | 44                     | 13                                 | 108                                   | 83                                                                     | 77                                        | 59                                                 | 0.85                             | 0.69               |
| Apollo 11, 12 fines | TINCLES             | 20–100   | $\sim 7 \times 10^6$                        | 154                    | 25                                 | 136                                   | 111                                                                    | 270                                       | 252                                                | 1.1                              | 2.3                |

\* Mean recordable track lengths in  $\mu\text{m}$  (one standard deviation  $\leq 1.5 \mu\text{m}$ ) in pyroxenes of interest.

|                                  | Cosmic ray nuclei |           |       |             |                   | Fission fragments                                 |             |         |       |
|----------------------------------|-------------------|-----------|-------|-------------|-------------------|---------------------------------------------------|-------------|---------|-------|
|                                  | Chromium          | Manganese | Iron  | Cobalt      | Nickel and copper | $^{238}\text{U}, ^{235}\text{U}, ^{244}\text{Pu}$ | Super-heavy |         |       |
| Length bracket ( $\mu\text{m}$ ) | 4–7               | 7–10      | 10–13 | 13–16       | 16–22             |                                                   | Z = 114     | Z = 126 |       |
| Mean length ( $\mu\text{m}$ )    | 5.5               | 8.5       | 11.5  | 15          | —                 | Length bracket ( $\mu\text{m}$ )                  | 13–16       | 16–22   | 19–24 |
| Relative contributions           | 0.25              | 0.5       | 1.0   | $\leq 0.25$ | $\leq 0.18$       |                                                   |             |         |       |

**Table 3** Fission Contributions to Tracks due to  $^{238}\text{U}$  and  $^{244}\text{Pu}$  in Meteorites and in Apollo "Fines"

| Sample              | Fossil $\rho$ due to CR and (U + Pu) fission ( $\text{cm}^{-2}$ ) | Estimated * $\rho$ due to (U + Pu) fission ( $\text{cm}^{-2}$ ) | Conc. of $^{238}\text{U}$ (ppb (wt.))† | Estimated $\rho$ due to spontaneous fission of $^{238}\text{U}$ in $4.5 \times 10^9$ yr ( $\text{cm}^{-2}$ ) | Estimated $^{244}\text{Pu}$ contribution $\rho(^{244}\text{Pu})/\rho(^{238}\text{U})$ ( $\text{cm}^{-2}$ ) |
|---------------------|-------------------------------------------------------------------|-----------------------------------------------------------------|----------------------------------------|--------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------|
| Angra dos Reis      | $12.3 \times 10^6$                                                | $12.3 \times 10^6$                                              | 110 (1,000)                            | $62 \times 10^4$                                                                                             | $11.7 \times 10^6$ 19                                                                                      |
| Moore County        | $3.0 \times 10^6$                                                 | $1.2 \times 10^6$                                               | 5.5 (268)                              | $3.1 \times 10^4$                                                                                            | $1.2 \times 10^6$ 39                                                                                       |
| Norton County       | $1.7 \times 10^5$                                                 | $0.39 \times 10^5$                                              | $< 0.3$ ‡                              | $< 0.17 \times 10^4$                                                                                         | $3.7 \times 10^4$ > 22                                                                                     |
| Kapoeta             | $3.0 \times 10^6$                                                 | $0.9 \times 10^6$                                               | 1.3 (47)                               | $0.74 \times 10^4$                                                                                           | $0.9 \times 10^6$ 120                                                                                      |
| Apollo 11, 12 fines | $7.0 \times 10^6$                                                 | $1.2 \times 10^6$                                               | 8.6 (60)                               | $4.85 \times 10^4$                                                                                           | $1.2 \times 10^6$ 25                                                                                       |

\* Subtracting contributions due to cosmic ray nuclei; see text and Table 2.

† Number of neutron-induced tracks counted are given within parentheses.

‡ Fleischer *et al.* (1967); reference No. 12.  
(CR refers to cosmic ray iron group nuclei.)



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- <sup>1</sup> Nilsson, S. G., Nix, J. R., Sobiczewski, A., Szymanski, Z., Wycech, S., Gustafson, C., and Moller, P., *Nucl. Phys.*, A, **115**, 545 (1968).
- <sup>2</sup> Nix, J. R., *Phys. Lett.*, **30**, B, 1 (1969).
- <sup>3</sup> Anders, E., and Heymann, D., *Science*, **164**, 821 (1969).
- <sup>4</sup> Dakowski, M., *Earth Planet. Sci. Lett.*, **6**, 152 (1969).
- <sup>5</sup> Price, P. B., and Fleischer, R. L., *Phys. Lett.*, **30**, B, 246 (1969).
- <sup>6</sup> Flerov, G. N., and Pereygin, V. P., *Sov. Atom. Energy*, **26**, No. 6, 603-605 (1969); also report D6-4649, *Search for Very Far Transuranium Elements in Iron-Manganese Nodules* (JINR, Dubna, 1970).
- <sup>7</sup> Price, P. B., Fleischer, R. L., and Woods, R. T., *Phys. Rev.*, C, **1** (5), 1819 (1970).
- <sup>8</sup> Cheifetz, E., Giusti, E. R., Bowman, H. R., Jared, R. C., Hunter, J. B., and Thompson, S. G., *Search for Superheavy Elements in Nature by Detection of Events with Large Number of Neutrons*, Univ. California, Berkeley Report UCRL-19957 (1970).
- <sup>9</sup> Bhandari, N., Bhat, S. G., Lal, D., Rajagopalan, G., Tamhane, A. S., and Venkatavaradan, V. S., *Apollo 12 Conf.*, **3**, Houston (1971).
- <sup>10</sup> Fleischer, R. L., Price, P. B., and Walker, R. M., *Geochim. Cosmochim. Acta*, **32**, 21 (1968).
- <sup>11</sup> Fleischer, R. L., Price, P. B., Walker, R. M., and Maurette, M., *J. Geophys. Res.*, **72**, 331 (1967).
- <sup>12</sup> Fleischer, R. L., Price, P. B., Walker, R. M., Maurette, M., and Morgan, G., *J. Geophys. Res.*, **72**, 355 (1967).
- <sup>13</sup> Lal, D., MacDougall, J. D., Wilkening, L., and Arrhenius, G., *Geochim. Cosmochim. Acta*, **3**, 2295 (1970).
- <sup>14</sup> Lal, D., *Space Sci. Rev.*, **9**, 623 (1969).
- <sup>15</sup> Lal, D., Rajan, R. S., and Tamhane, A. S., *Nature*, **221**, 33 (1969).
- <sup>16</sup> Henke, R. P., and Benton, E. V., *A Computer Code for the Computation of Heavy Ion Range Energy Relationships in any Stopping Material*, Report USNRDL-TR-67-122 (1967).
- <sup>17</sup> Hyde, E. K., *Nuclear Properties of Heavy Elements*, **3**, 171 (Prentice-Hall, London, 1964).
- <sup>18</sup> Viola, V. E., and Seaborg, G. T., *J. Inorg. Nucl. Chem.*, **28**, 697 (1966).
- <sup>19</sup> Fleischer, R. L., Price, P. B., Walker, R. M., Filz, R. C., Fukui, K., Friedlander, M. W., Holeman, E., Rajan, R. S., and Tamhane, A. S., *Science*, **155**, 187 (1967).
- <sup>20</sup> Price, P. B., Fleischer, R. L., and Moak, C. D., *Phys. Rev.*, **167**, 277 (1968).
- <sup>21</sup> Shirck, J., Hoppe, M., Maurette, M., and Walker, R. M., *Meteorite Research*, 41 (Reidel, Dordrecht, 1969).
- <sup>22</sup> Rao, M. N., *Nucl. Phys.*, A, **140**, 69 (1970).

# Extensions of the Allosteric Model for Haemoglobin

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**So far it has not been possible to distinguish experimentally between two possible models for cooperative ligand binding by haemoglobin. Numerical analysis, however, yields a unique value for the allosteric constant showing that only the two state model can account for the behaviour of the protein.**

A GREAT deal of information is now available about the structure of haemoglobin, largely from the X-ray studies of Perutz<sup>1</sup>. The differences in quaternary conformation of the liganded and unliganded forms of haemoglobin have been specified and many of the residues which are involved in the conformational change have been identified. These structural studies provide a clear picture of many of the atomic movements accompanying ligand binding. Certain dynamic aspects of the cooperative ligand binding by haemoglobin, however, remain uncertain. According to the allosteric model by Monod *et al.*<sup>2</sup> cooperativity would arise simply from the adjustment of an equilibrium between the two conformational states observed by Perutz. But alternative models have been described, notably the sequential model proposed by Pauling in 1935<sup>3</sup> and favoured by Koshland *et al.*<sup>4</sup> in which conformational changes occur in steps during ligand binding. The two state and sequential models differ in several details, but from the point of view of

fundamental processes, the chief distinction concerns the existence of conformational equilibria. The treatment by Monod *et al.* requires a pre-existing conformational isomerization equilibrium which is perturbed by ligand. The Koshland approach is based on induced fit whereby changes in conformation only occur after ligand binding.

Several experimental approaches have been reported and interpreted to exclude one model and indirectly imply the other; the most significant fit into two categories: (1) tests for the deviations from linearity in the response of some structural parameter to the addition of ligand as predicted by the two state model in certain conditions; (2) experiments which seek to detect the conformational equilibrium by attempting to measure the small amounts of one conformation postulated to exist by the two state model, in conditions which greatly favour the other. For haemoglobin, a typical experiment of the first type was the spin-label study of Ogawa and McConnell<sup>5</sup>. Linearity of the spin-label change to oxygenation was reported, whereas the behaviour predicted from calculations with the two state model was distinctly non-linear. The results were therefore interpreted as implying a sequential model. The second type of experiment is illustrated by work with the haptoglobin-haemoglobin system. Haptoglobin binds very tightly to oxyhaemoglobin but generally not at all to deoxyhaemoglobin<sup>6,7</sup>. Because the conformational isomerization of the two state model requires some small amount of the oxy-like material in the deoxyhaemoglobin solutions (which should bind haptoglobin) the failure to observe binding has been cited as evidence against the two state model<sup>8</sup>. Conclusions of both types of experiment, however, rely heavily on the value

of the conformational isomerization equilibrium constant used. This constant,  $L$  in the nomenclature of Monod *et al.*<sup>2</sup>, has been estimated in all cases only by casual fitting. We wish to describe a more rigorous method for evaluating  $L$  based on a consideration of the properties of both haemoglobin and its isolated chains. Values of  $L$  much larger than earlier estimates are obtained by this procedure, and the revised values bear critically on the several experiments designed to distinguish models.

The second part of this article concerns the incorporation of the new values of  $L$  in an analysis of the cooperativity of haemoglobin in various conditions for normal and mutant haemoglobins. Efforts at accommodating this set of ligand binding curves with both the two state and sequential models are described.

## Size of Allosteric Constant for Haemoglobin

The basis of the two state model is an equilibrium between the oligomeric states called R and T. The relaxed state, R, displays high affinity for ligand (given by the dissociation constant  $K_R$ ) and the constrained state exhibits low affinity for ligand (given by  $K_T$  where  $K_R < K_T$  and  $c = K_R/K_T$ ). The isomerization equilibrium is described by the equilibrium constant  $L$ , where  $L = [T]/[R]$  in the absence of ligand. The term  $L$  is also referred to as the allosteric constant. Monod *et al.*<sup>2</sup> originally reported a value for haemoglobin under standard conditions of  $L = 9 \times 10^3$  and  $c = 0.014$ . The values are obtained<sup>2</sup> by fitting oxygen saturation data to the equation

$$Y = \frac{\alpha(1+\alpha)^3 + Lc\alpha(1+\alpha)}{(1+\alpha)^4 + L(1+\alpha)} \quad (1)$$

where  $Y$  is the fractional saturation and  $\alpha$  is a normalized ligand concentration ( $\alpha = pO_2/K_R$ ). A detailed analysis of the fitting of this equation to the oxygen binding properties of haemoglobin yields two important points. First, the values of  $L$  and  $c$  reported by Monod *et al.*<sup>2</sup> are not unique and widely differing combinations can also represent the data, particularly since  $K_R$  is not specified (Fig. 1). Two theoretical curves with differences that fall within usual experimental errors are shown to represent the ligand binding by haemoglobin. The values of  $L$  for the two curves differ greatly:  $2 \times 10^3$  compared with  $3 \times 10^5$ . The second point, however, is that a unique solution to the two state equation for haemoglobin is possible by considering the properties of the isolated chains.

By virtually all criteria, the isolated  $\alpha$  and  $\beta$  chains of haemoglobin possess the same, or very nearly the same, conformation as the individual chains in liganded  $\alpha_2\beta_2$  haemoglobin. The criteria include spectral similarities<sup>9</sup> and agreement in kinetic properties between chains<sup>10</sup> and the short lived "rapid" haemoglobin observed in the Gibson effect<sup>11</sup>. In the one exception<sup>12</sup>, the differences are only minor. Therefore, if chains and the R state are effectively identical, the ligand affinity of the chains is an indicator of the affinity of the R state and defines  $K_R$ . Since chains are apparently locked in the high affinity state, equation (1) reduces to equation (2)

$$Y = \frac{\alpha}{1+\alpha} \quad (2)$$

and at half saturation  $\alpha_1 = 1.0$ . Thus as a consequence of the definition of  $\alpha$  a scale for haemoglobin is provided with  $\alpha_1$  for chains set at unity and the relative affinity of haemoglobin set accordingly. For example, the affinity of normal human haemoglobin at pH 7 is about twenty-four to thirty times less than isolated chains<sup>13</sup>. The  $\alpha_1$  must therefore fall in the range of twenty-four to thirty.

With  $\alpha_1$  fixed, the values of  $L$  and  $c$  may be considered. First, the constant  $L$  is directly related to  $\alpha_1$ . Chains are characterized by  $\alpha_1 = 1$ , so the higher values of  $\alpha_1$  observed for native

haemoglobin are linked to the relative stability of the T state: the more stable the T state (that is, the larger the value of  $L$ ), the higher the value of  $\alpha_1$ . Because four molecules of ligand are bound by each molecule of haemoglobin, the exact linkage relationship takes the form  $L = (\alpha_1)^4$ . A more complete development of these relationships will be presented later, when the linkage to subunit interactions will also be considered. With a minimum value of  $\alpha$  set at 24, a minimum value of  $L = 3 \times 10^5$  is obtained. Any lower value of  $L$  will yield a predicted ligand binding curve with an affinity too close to that of chains. With  $L = (\alpha_1)^4$ , the cooperativity as reflected by the Hill constant,  $n$ , is set by  $c$ . For  $L = 3 \times 10^5$ ,  $n = 2.8$ , the usual experimental value for haemoglobin, when  $c = 0.01$ . Higher values of  $c$  lower  $n$  and diminish cooperativity.

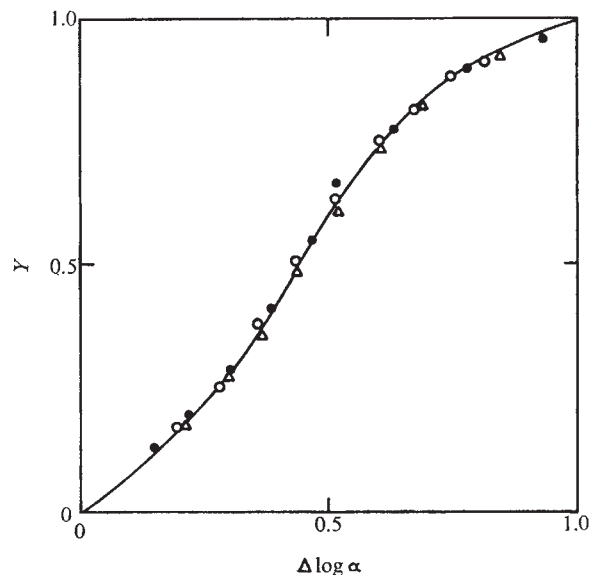
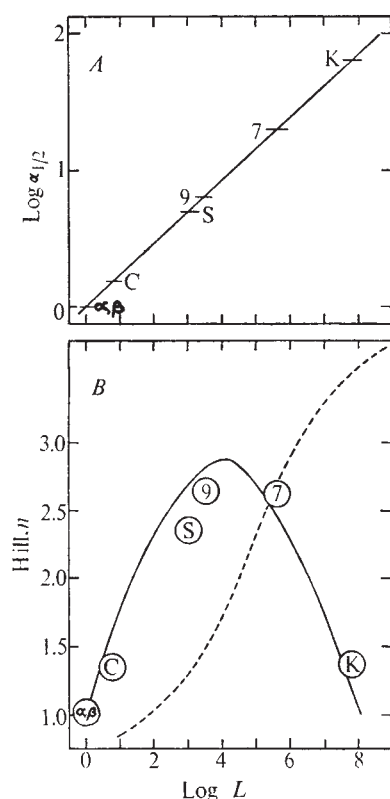


Fig. 1 Representative oxygen binding for haemoglobin. Fractional ligand binding ( $Y$ ) versus the logarithm of the relative ligand affinity. The points (●) represent a ligand binding curve generated from the empirical Adair equation using values for the four phenomenological equilibrium constants described by Gibson<sup>15</sup>. These values are in good agreement with early gasometric determinations. For these data, zero on the abscissa corresponds to  $\log pO_2 = 0.6$ . Two additional sets of points are provided, calculated with the two state model (equation 1). For the points represented by (Δ) a value of  $L = 3 \times 10^5$  was used. The points represented by (○) are obtained with  $L = 2 \times 10^3$ . The two theoretical curves are on shifted scales, corresponding to different values for the unspecified parameter  $K_R$ . For the curve with  $L = 3 \times 10^5$ , zero on the abscissa is equal to a value of  $\log \alpha = 0.9$ , and for  $L = 2 \times 10^3$ ,  $\log \alpha = 0.4$ .

In the simple formulation of the two state model, no intermediate states are considered. If intermediate states occur, our analysis still applies in terms of  $L$ . But intermediates destabilize cooperative effects and as a consequence the value of  $c$  must be diminished to maintain the Hill constant at  $n = 2.8$ . For example, if one intermediate state, I (half R, half T), is added to the two state formulation, the proper equations are easily derived<sup>14</sup> and a new parameter  $L_1$  appears ( $L_1 = [I]/[R]$  in the absence of ligand). In this case, a good representation of the haemoglobin saturation curve is obtained with  $L_1 = 750$  and  $c = 0.001$ . The value of  $L$  remains fixed at  $3 \times 10^5$ . This particular set of parameters was selected, since the kinetic data of Gibson<sup>15</sup> suggest a value of  $c$  close to 0.001. The presence of an intermediate state would also account for the two stage process implied by Gibson<sup>15</sup>. Thus, the magnitude of an intermediate term,  $L_1$ , may prove some measure of the extent to which the two state model is an approximation and inter-





**Fig. 2** Composite ligand binding properties for haemoglobin. *A*, Dependence of the logarithm of ligand binding affinity on the logarithm of the allosteric constant. The line was obtained by generating a series of theoretical ligand binding curves with equation (1) with  $c=0.01$  and plotting  $\log \alpha_1$  versus  $\log L$ . The straight line resulted. The various examples of haemoglobin: isolated chains ( $\alpha+\beta$ ), Chesapeake (C), stripped (S), normal at pH 7 (7), normal at pH 9 (9) and Kansas (K), were then located on the affinity curve in terms of their measured properties relative to the isolated chains. *B*, Dependence of cooperativity (the Hill constant,  $n$ ) on  $\log L$ . For the series of theoretical curves used to generate  $\log \alpha_1$  versus  $\log L$  in *A*, the overall cooperativity was also estimated. The average value of  $n$ , where  $n=d \log (Y/(1-Y))/d \log \alpha$ , between  $Y=0.25$  and  $Y=0.75$  was calculated. When these values of  $n$  are plotted against  $\log L$ , the bell shaped curve is obtained. The six points circled, representing the examples of haemoglobin, were located horizontally on the curve according to the value of  $\log L$  indicated in *A*. The vertical position corresponding to cooperativity was determined by the published values from equilibrium measurements (see text). The dashed line represents values of  $n$  calculated for the sequential model, using equation (13) of Koshland *et al.*<sup>4</sup>. Values of  $K_{BB}$  and  $K_{AB}$  were selected to represent the observed affinity and cooperativity of haemoglobin at pH 7.0. The general dependence of cooperativity on  $\log L$  (equivalent to  $\log K_{BB}$ ) was then determined by generating binding curves with different values of  $K_{BB}$ .

mediate states may have to be included. However, stripped haemoglobin, which is perhaps more easily interpreted kinetically because factors related to phosphate binding can be ignored, yields values closer to  $c=0.01$  (ref. 15) and is therefore less suggestive of intermediates.

## Conformational Equilibria and Cooperativity

With the quantitative procedure for obtaining the value of the allosteric constant,  $L$ , described earlier in this article, it is now possible to compare the behaviour of haemoglobin in various conditions, as well as mutant haemoglobins, in terms of mechanistic models. One of the most salient features of the two state model is the "buffering" of cooperativity caused by the conformational equilibrium. As first pointed out by Rubin and Changeux<sup>16</sup> cooperativity is relatively independent of  $L$  for

values of  $L$  near  $c^{-1/2}$  (where  $i$  is the number of binding sites). The overall dependence of cooperativity on  $L$  is bell shaped with the Hill constant,  $n$ , diminishing at both high and low values of  $L$ . This property of the two state model was emphasized by Rubin and Changeux<sup>16</sup> to accommodate the Bohr effect in haemoglobin—the change in affinity with little or no change in cooperativity observed with changing pH. The dependence of cooperativity and affinity on  $L$  is further explored in this paper encompassing a wider range of haemoglobin ligand binding data.

The general dependence of  $\alpha_1$  and  $L$  is illustrated in Fig. 2*A*. The values of  $\log \alpha_1$  vary linearly with  $\log L$ , with  $\alpha_1=1$ , corresponding to haemoglobin chains. Haemoglobin at pH 7 and pH 9 (ref. 17), stripped haemoglobin<sup>19</sup> and the mutant haemoglobins Kansas<sup>19</sup> and Chesapeake<sup>20</sup> can therefore be placed on the line of  $\log \alpha_1$  against  $\log L$  according to their measured affinities. A unique value of  $L$  is thereby specified for each haemoglobin. With  $L$  fixed, each haemoglobin is also located on the bell shaped curve of the Hill constant  $n$  versus  $\log L$ . As seen in Fig. 2*B*, the reported values for  $n$  for each haemoglobin come very close to the calculated line. The data for pH 7 and pH 9 lie near the maximum of the curve accounting for the invariance of cooperativity accompanying affinity changes in the Bohr effect. Presumably the value of  $L$  changes in these regions because of the changes in the differential binding of protons by the R and T states (ref. 22 gives a more detailed explanation). The value for stripped haemoglobin also coincides approximately with the predicted curve, although the exact degree of cooperativity is not certain because some differences exist in the measured values<sup>15,18</sup>. The most interesting results, however, involve the mutant haemoglobins Chesapeake and Kansas. These two species represent a symmetric case of alterations in the  $\alpha_1$ - $\beta_2$  interface of haemoglobin. In haemoglobin Chesapeake the change (leu FG4(92)  $\alpha \rightarrow \arg$ ) results in enhanced stability of the liganded tetramer as evidenced by diminished dissociation of oxyhaemoglobin (ref. 22 and R. Nagel, Q. H. Gibson and S. J. E., in preparation). In contrast haemoglobin Kansas (asn G4(102) $\beta \rightarrow \text{tyr}$ ) tends to dissociate more readily in the liganded form<sup>19</sup>. It is possible to demonstrate that the displacement of  $L$  for these mutants (Fig. 2*B*) corresponds quite well with the changes in the subunit dissociation, according to linkage principles (S. J. E., in preparation). Most significant, however, is the fact that both increases and decreases in stabilization diminish cooperativity to about  $n=1.3$  in the two cases. This property is in striking agreement with the bell shaped dependence of cooperativity on  $L$  as shown in Fig. 2 and as predicted by a model based on conformational equilibrium. As will now be shown the sequential or induced fit model leads to a contrary prediction for these two mutants and poorly accommodates the Bohr effect.

Just as the value of  $L$  in the two state model is uniquely determined by the ligand binding affinity, so, too, is the principal parameter of the sequential model,  $K_{BB}$  in the nomenclature of Koshland *et al.*<sup>4</sup>. With the sequential model  $K_{BB}$  relates to the change in subunit bonding induced by the binding of ligand. The term  $K_{BB}$  is defined with respect to a single subunit interface, so that as many as six binding surfaces could be involved in a molecule such as haemoglobin, with pseudotetrahedral symmetry. As can be shown from linkage principles (S. J. E., in preparation), the dependence of  $K_{BB}$  on ligand binding affinity relative to isolated chains for haemoglobin gives a linear relationship of  $\log \alpha_1$  (or the ligand concentration at half saturation) versus  $\log (K_{BB})^6$ . This relationship is identical to the dependence shown in Fig. 2*A*. In other terms  $(K_{BB})^6$  is equivalent to  $L$  in that both are fixed according to linkage principles by the affinity of haemoglobin compared with its isolated chains:  $L=(\alpha_1)^4$  and  $K_{BB}^6=(\alpha_1)^4$ .

With  $K_{BB}$  defined for the set of haemoglobins considered in Fig. 2, the question of cooperativity may now be considered. With the two state model, only  $c$  is freely adjustable to establish the proper cooperativity and a value of  $c=0.01$  was

selected to fit the behaviour at pH 7.0. This value then represented the other members of the haemoglobin set in conjunction with the values of  $L$  fixed by their respective affinities. For the sequential model the parameter  $K_{AB}$  is free to adjust to the degree of cooperativity. The term  $K_{AB}$  relates to the hybrid subunit bonding. In the sequential scheme, each fractionally saturated intermediate contains subunits in different conformations (liganded and unliganded) and  $K_{AB}$  relates to their interactions. In contrast,  $K_{BB}$  is dependent only on the relative bonding of a pair of liganded subunits compared with a pair of unliganded subunits. Because  $K_{AB}$  defines the strength and hence the relative occurrence of intermediates, the larger  $K_{AB}$ , the more intermediates and the lower the cooperativity. Conversely, as  $K_{AB}$  goes to zero, cooperativity or the Hill constant  $n$  tends to the maximum defined by the number of ligand binding sites. For haemoglobin at pH 7 the affinity corresponds to a value of  $K_{BB}=1/9$  and the cooperativity corresponds to a value of  $K_{AB}=2/3$ . The change in cooperativity with change in affinity is given in Fig. 2B by the dashed line. The values for the sequential model were selected to coincide with the two state model near pH 7.0. But the sequential model, unlike the two state model, predicts a continuous increase in cooperativity with rising  $\alpha_4$  (or log  $L$  which in turn is equivalent to log  $K_{BB}$ <sup>6</sup>). The behaviour of haemoglobin Kansas therefore falls far off the dashed curve. Moreover, the sequential model predicts a much steeper dependence of the changes in cooperativity with changing affinity than predicted by the two state model. The values of the Hill constant for stripped haemoglobins, haemoglobin at pH 9 and haemoglobin Chesapeake are thus much more poorly represented by the sequential model than with the two state model. The sequential model can fit the Hill constant for each haemoglobin in the set, but only by making arbitrary changes in the term  $K_{AB}$ . Its value must be lowered for haemoglobin Chesapeake and stripped haemoglobin, markedly raised for haemoglobin Kansas and altered in such a way as to just compensate for the invariance of cooperativity in the Bohr effect. A physical basis for such a pattern of changes is difficult to imagine. Thus, in terms of their ability to represent the ensemble of haemoglobin data, the two state model is very successful, whereas the sequential model requires a number of arbitrary adjustments.

## Value and Limitations of Numerical Models

The molecular action of haemoglobin is complex and a battery of physical measurements will be required to unfold the intricate details. Dynamic techniques such as rapid reaction kinetics<sup>16</sup> have already provided much important information and in conjunction with the X-ray structures hold out the hope of a complete understanding at some future time. Eventually, they should provide more direct evidence about the applicability of the two state, sequential or some other model for haemoglobin. But it is worth emphasizing the value of some simple numerical exercises as described here. Even the more complex physical studies require values for the principal parameters of the competing models to orient the analysis of their data and a new method has been presented for obtaining these values in a more rigorous way than previously available. One result of the new method is to raise the value of the allosteric constant,  $L$ , for haemoglobin, from  $10^4$  to  $3 \times 10^5$ . The higher value should require a reconsideration of some of the experimental studies which have claimed to dismiss the presence of a conformational equilibrium<sup>8</sup>. Furthermore, a lower value of  $L$  (about  $10^3$ ) is now indicated by the relationship  $L=(\alpha_4)^4$  for the spin labelled haemoglobin of Ogawa and McConnell<sup>5</sup>; the new value of  $L$  predicts that the R state and binding functions should be effectively coincident, as is in fact observed. The numerical exercises presented here are especially striking in terms of the haemoglobin data for the Bohr effect and mutants with high and low affinity (Fig. 2). The behaviour for all these cases is predicted by the two state model,

once values of the constants are established for the "standard" data at pH 7.0. In marked contrast the values of the constants for the sequential model when rigorously set for pH 7.0 fail to accommodate the other haemoglobin data. Only by ancillary adjustments can all the data be accommodated. These comparisons cannot rule out the sequential model, but seriously weaken its validity.

In any consideration of models, it should be kept in mind that in many respects both the two state and sequential treatments must be gross simplifications. They can, of course, distinguish fundamental processes, such as the presence and absence of conformational equilibria. But, beyond this stage, there is an enormous body of detail concerning, for example, the exact kinetic rate for each process<sup>15</sup>. A knowledge of the structural basis of functional parameters such as  $L$  will also eventually be required. In this respect some information can be deduced from the subunit association-dissociation equilibria of native haemoglobin and its mutants. Preliminary examination indicates a close parallel between changes in these subunit equilibria measured in the analytical ultracentrifuge<sup>23</sup> and predicted value of  $L$  (Fig. 2B) when correlated by linkage principles<sup>14</sup>.

In conclusion, this presentation emphasizes the likely presence of conformational equilibria. Whether or not only two states are involved in this equilibria is beyond the scope of this simple analysis. Conceivably an additional state, such as the half R-half T intermediate might be present, with marked implications for details of the kinetic or spectroscopic studies. Even with the addition of this state, model calculations show that results broadly the same as seen in Fig. 2 will be obtained. Therefore, an analysis of overall affinity and cooperativity properties can tend to exclude certain types of models such as the induced fit sequential treatment and favour conformational equilibria, but little can be said concerning the precise mechanism whereby the equilibria are expressed. Rather, information from dynamic experiments (see, for example, ref. 15) must provide these details.

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- <sup>1</sup> Perutz, M. F., *Proc. Roy. Soc., B*, **173**, 113 (1969).
- <sup>2</sup> Monod, J., Wyman, J., and Changeux, J. P., *J. Mol. Biol.*, **12**, 88 (1965).
- <sup>3</sup> Pauling, L., *Proc. US Nat. Acad. Sci.*, **21**, 186 (1935).
- <sup>4</sup> Koshland, D. E., Nemethy, G., and Filmer, D., *Biochemistry*, **5**, 365 (1966).
- <sup>5</sup> Ogawa, S., and McConnell, H. M., *Proc. US Nat. Acad. Sci.*, **58**, 19 (1967).
- <sup>6</sup> Nagel, R. L., Wittenberg, J. B., and Ranney, H. M., *Biochim. Biophys. Acta*, **100**, 286 (1965).
- <sup>7</sup> Chiancone, E., Wittenberg, J. B., Wittenberg, B. A., Antonini, E., and Wyman, J., *Biochim. Biophys. Acta*, **117**, 379 (1966).
- <sup>8</sup> Antonini, E., and Brunori, M., *Ann. Rev. Biochem.*, **39**, 977 (1970).
- <sup>9</sup> Antonini, E., Bucci, E., Fronticelli, C., Wyman, J., and Rossi-Fanelli, A., *J. Mol. Biol.*, **12**, 375 (1965).
- <sup>10</sup> Noble, R. W., Gibson, Q. H., Brunori, M., Antonini, E., and Wyman, J., *J. Biol. Chem.*, **244**, 3905 (1969).
- <sup>11</sup> Gibson, Q. H., *Biochem. J.*, **71**, 293 (1959).
- <sup>12</sup> Shulman, R. G., Ogawa, S., Wuthrich, K., Yamane, T., Peisach, J., and Blumberg, W. E., *Science*, **165**, 251 (1969).
- <sup>13</sup> Noble, R. W., *J. Mol. Biol.*, **39**, 479 (1969).
- <sup>14</sup> Edelstein, S. J., thesis, Univ. California (1967).
- <sup>15</sup> Gibson, Q. H., *J. Biol. Chem.*, **245**, 3285 (1970).
- <sup>16</sup> Rubin, M. M., and Changeux, J. P., *J. Mol. Biol.*, **21**, 265 (1966).
- <sup>17</sup> Roughton, F. J. W., and Lyster, R. L. J., *Hvalradets Skrifter*, **48**, 185 (1965).
- <sup>18</sup> Benesch, R. E., Benesch, R., and Yu, C. I., *Biochemistry*, **8**, 2567 (1969).
- <sup>19</sup> Bonaventura, J., and Riggs, A. J., *Biol. Chem.*, **243**, 980 (1968).
- <sup>20</sup> Nagel, R. L., Gibson, Q. H., and Charache, S., *Biochemistry*, **6**, 2395 (1967).
- <sup>21</sup> Perutz, M. F., Muirhead, H., Mazzarella, L., Crowther, R. A., Greer, J., and Kilmartin, J. V., *Nature*, **222**, 1240 (1969).
- <sup>22</sup> Bunn, H. F., *Nature*, **227**, 839 (1970).
- <sup>23</sup> Edelstein, S. J., Rehmar, M. J., Olson, J. S., and Gibson, Q. H., *J. Biol. Chem.*, **245**, 4372 (1970).



# Identification of 1,25-Dihydroxycholecalciferol, a New Kidney Hormone controlling Calcium Metabolism

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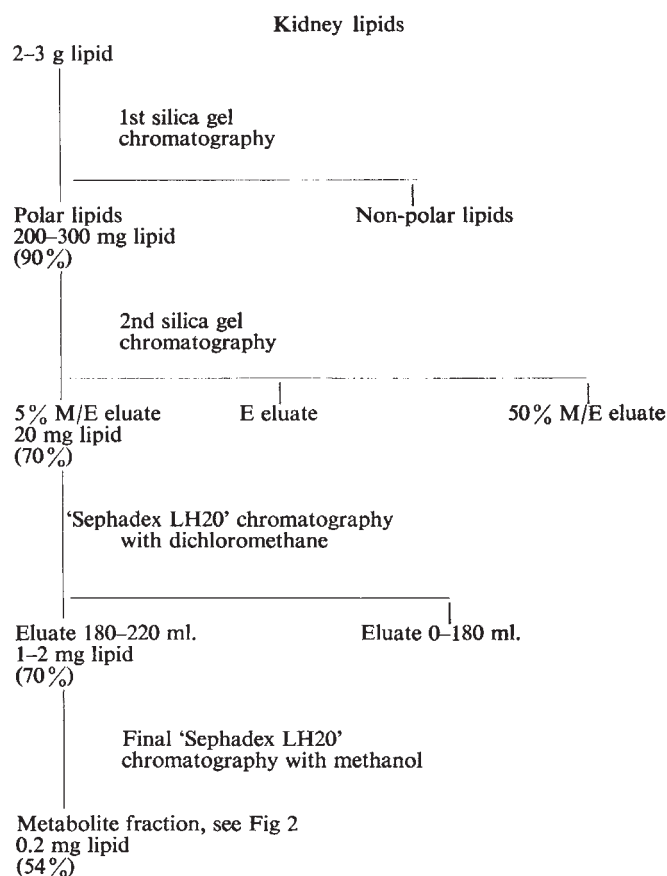
**A vitamin D metabolite, 25-dihydroxycholecalciferol, is further hydroxylated by kidney before acting as a hormone on target tissues. The structure of this kidney metabolite is now described.**

It has been established that cholecalciferol undergoes several metabolic transformations before it can affect calcium metabolism in its target tissues such as intestine and bone<sup>1-5</sup>. In particular, a metabolite is produced by the kidney which seems to be obligatory for vitamin D activity<sup>6</sup>. We have identified this metabolite, and suggest that it should be classified as a hormone.

Previous reports from this laboratory have shown that after intravascular injection of small amounts of [4-<sup>14</sup>C, 1,2-<sup>3</sup>H]-cholecalciferol<sup>7</sup> and [4-<sup>14</sup>C, 1-<sup>3</sup>H]-cholecalciferol<sup>2</sup>, a polar metabolite of vitamin D was formed (designated peak P) which had lost <sup>3</sup>H from C-1 only, and has been found in several species including the chick<sup>2</sup>, rat<sup>7</sup>, pig<sup>8</sup>, sheep and man (D. E. M. L., D. R. F. and E. K., unpublished observations). The highest concentrations of this metabolite occur in intestine and bone, and it is specifically accumulated in mucosal cell nuclei of the small intestine<sup>2,4</sup>. 25-Hydroxycholecalciferol, a metabolite of vitamin D formed in the liver<sup>9</sup>, is an intermediate in the biosynthesis of peak P from cholecalciferol<sup>2</sup>. A bioassay of peak P derived from chick intestines showed three times as much activity as cholecalciferol in stimulating the intestinal absorption of <sup>45</sup>Ca in vitamin D-deficient chicks<sup>5</sup>. Investigations into the role of this metabolite on bone metabolism await the availability of larger quantities of this steroid.

Examining the biosynthesis of peak P, Fraser and Kodicek<sup>6</sup> have demonstrated the key role of the kidney in the metabolism of 25-hydroxycholecalciferol. After an exhaustive search for possible biosynthetic sites, it was found that only kidney converted [4-<sup>14</sup>C, 1-<sup>3</sup>H]-25-hydroxycholecalciferol *in vitro* into a polar metabolite with concomitant loss of <sup>3</sup>H. This polar

metabolite co-chromatographed on two thin-layer systems with the metabolite found in the intestine. Further, removal of the kidneys from rachitic rats prevented the formation *in vivo*



**Fig. 1** Scheme of isolation of tritium-deficient metabolite from the lipid extract of kidney homogenates incubated with 26-<sup>14</sup>C, [1-<sup>3</sup>H]-25-hydroxycholecalciferol. M, Methanol; E, diethyl ether; numbers in parentheses denote the percentage recovery of the metabolite originally present in kidney lipids.

of peak P from [4- $^{14}\text{C}$ , 1- $^3\text{H}$ ]-cholecalciferol. Therefore 25-hydroxycholecalciferol is metabolized in the kidney into either the intestinal peak P or a precursor polar metabolite which, after transport to the target tissue, could be converted into peak P.

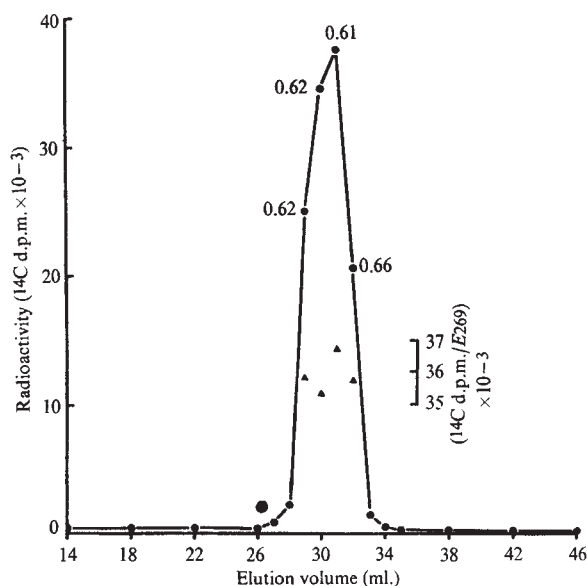


Fig. 2 Final chromatography with methanol on 'Sephadex LH20' of lipid from the incubation of [26- $^{14}\text{C}$ , 1- $^3\text{H}$ ]-25-hydroxycholecalciferol with vitamin D-deficient chick kidney homogenate.  $^3\text{H}/^{14}\text{C}$  specific radioactivity ratio figures are shown at each sampling point through the peak. The ratios of  $^{14}\text{C}$  d.p.m./ $E_{269}$  at the same points are indicated by  $\blacktriangle$ .

To identify peak P chemically from the small quantities found naturally in animal tissues (<3 ng/g) would require extraction of at least 1,000 kg of tissue to obtain sufficient material. But the synthesis of peak P of kidney by homogenates of this tissue provides a means of preparing readily sufficient quantities for chemical identification and biological study. [26- $^{14}\text{C}$ ]-25-hydroxycholecalciferol (34.8 mCi/mmol) was synthesized from 25-keto, 26-norcholecalciferol and  $^{14}\text{CH}_3\text{MgI}$  (in collaboration with Dr P. A. Bell). [1- $^3\text{H}$ ]-cholecalciferol (211 mCi/mmol) was administered to pigs and the [1- $^3\text{H}$ ]-25-hydroxycholecalciferol was isolated from the plasma. As enzyme substrate [26- $^{14}\text{C}$ , 1- $^3\text{H}$ ]-25-hydroxycholecalciferol was diluted with unlabelled 25-hydroxycholecalciferol to give specific activities for  $^{14}\text{C}$  and  $^3\text{H}$  of 0.36 mCi/mmol and 0.9 mCi/mmol respectively. The  $^3\text{H}/^{14}\text{C}$  specific radioactivity ratio was 2.5:1. In each preparation of metabolite, [26- $^{14}\text{C}$ , 1- $^3\text{H}$ ]-25-hydroxycholecalciferol at a concentration of  $2 \times 10^{-6}$  M was incubated under oxygen with a homogenate of kidney from twenty vitamin D-deficient chicks and an NADPH generating system as described previously<sup>6</sup>. Each 2 h incubation converted about 15% of the substrate to the tritium-deficient metabolite as assayed by formation of tritiated water and by the drop in the  $^3\text{H}/^{14}\text{C}$  ratio of the extracted radioactivity. The metabolite extracted from the homogenate with  $\text{CHCl}_3$ /methanol was purified as described in Fig. 1. The silica gel chromatograms have been described before<sup>2</sup>. The first 'Sephadex LH20' column (60  $\times$  1.5 cm) was eluted with dichloromethane. After collecting 180 ml. of eluate, the kidney metabolite with a low  $^3\text{H}/^{14}\text{C}$  ratio was recovered over the following 40 ml. This material was applied to a second 'Sephadex LH20' column (60  $\times$  1.0 cm) and developed with methanol. The  $^3\text{H}/^{14}\text{C}$  ratio and the  $^{14}\text{C}$  d.p.m./ $E$  at  $\lambda_{\text{max}}$  through the radioactive peak indicated that the material was radiochemically and spectroscopically pure (Fig. 2). In this way, 63  $\mu\text{g}$  of metabolite was

produced from two incubations with an overall yield of 54% during purification.

The mass spectrum of this metabolite, as both the free steroid and the trimethylsilyl (TMS) ether (Fig. 3) was compared with similar spectra of cholecalciferol, 25-hydroxycholecalciferol, isotachysterol and isocholecalciferol.

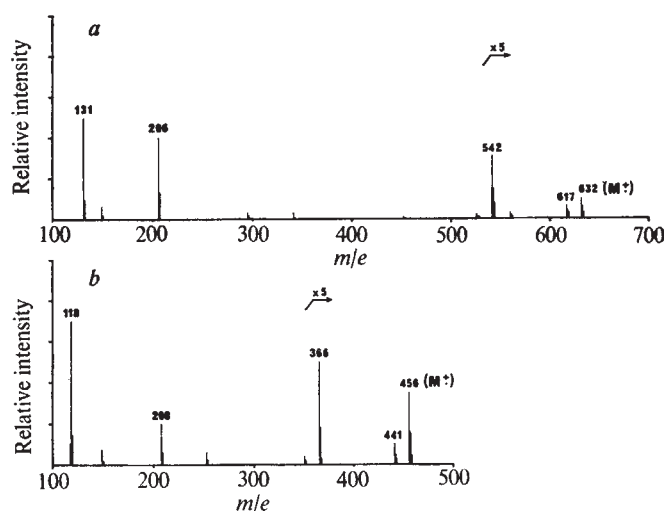


Fig. 3 Partial mass spectrum (above  $m/e$  100) of the TMS-derivatives of (a) peak P (source temperature  $175^\circ\text{C}$ ) and (b) cholecalciferol (source temperature  $140^\circ\text{C}$ ).

The molecular ions of the metabolite and its TMS ether at  $m/e$  416 and 632, respectively, established that two additional oxygen atoms had been inserted into the cholecalciferol molecule in the form of two hydroxyl groups.

Cleavage of the  $\text{C}_{24}$ - $\text{C}_{25}$  bond gave intense peaks at  $m/e$  59 and 131 in the spectra of the free steroid and the TMS ether respectively (Fig. 4), confirming the presence of the hydroxyl group at C-25. The spectrum of the free steroid has peaks at 287 and 269 corresponding to the loss of the side chain, followed

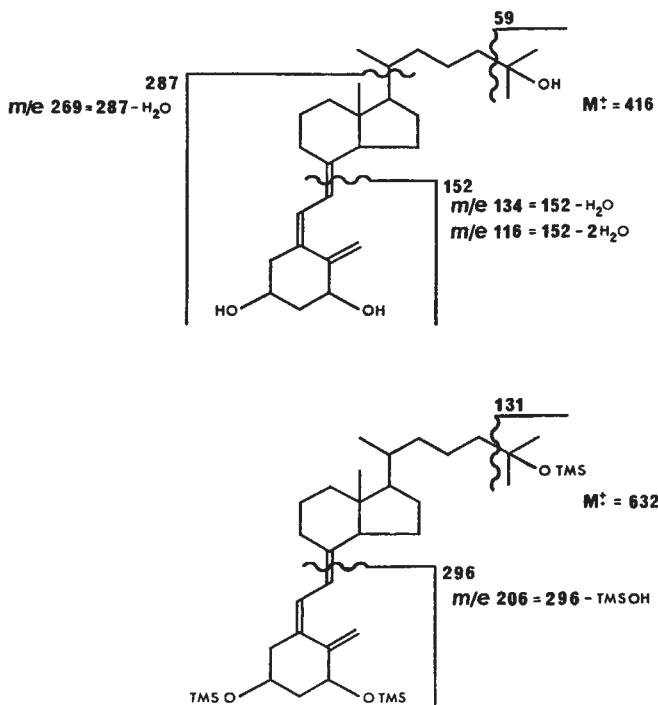


Fig. 4 Fragmentation of 1,25-dihydroxycholecalciferol and its tri-TMS derivative.



by the elimination of water. This indicates that the third hydroxyl group is in the steroid nucleus. The mass spectrum of cholecalciferol, 25-hydroxycholecalciferol, and all molecules so far examined with this conjugated triene system have intense peaks at  $m/e$  136 and 118 ( $m/e$  136 minus  $H_2O$ ), and in the case of their TMS ethers at  $m/e$  208 and 118 ( $m/e$  208 minus trimethylsilanol). The peaks at  $m/e$  136 and 208 have been attributed to the fragment formed by the A ring plus C-6, C-7 and C-19 (cleavage of  $C_7-C_8$  bond)<sup>10</sup>. The mass spectrum of the metabolite had intense peaks at  $m/e$  134 and 116, and that of the TMS ether derivative at  $m/e$  206. The mass spectrum of a mixture of the TMS ether and the deuteriated TMS ether of the metabolite had intense peaks at  $m/e$  206 and 215 ( $d_9$ -206) and confirmed that the peak at  $m/e$  206 was due to the A ring plus C-6, C-7 and C-19. We interpret the difference for peak P and cholecalciferol or their TMS derivatives, of two mass units between  $m/e$  134 and 136, or  $m/e$  206 and 208 as being caused by the elimination of water or trimethylsilanol respectively, suggesting that the third hydroxyl group is in the A ring plus C-6, C-7 and C-19. This typical vitamin D fragmentation pattern (not shown by, for example, isotachysterol and isocholecalciferol), observed for the metabolite and its TMS ether derivative, is consistent with retention of the  $\Delta^{5,7,10(19)}$  triene structure and the siting of the third hydroxyl group in the A ring itself rather than at C-6 or C-7.

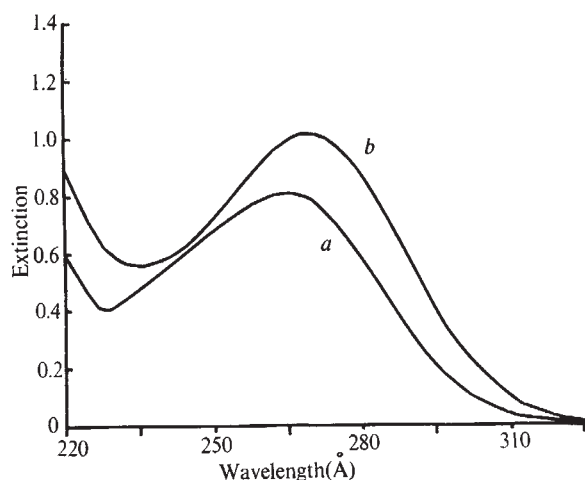


Fig. 5 Ultraviolet absorption spectra in ethanol of equimolar quantities of (a) cholecalciferol and (b) peak P.

The ultraviolet spectrum (Fig. 5) should have provided unequivocal evidence of the nature of the chromophore, but the metabolite exhibited a single peak with  $\lambda_{max}$  at 269 nm and  $\lambda_{min}$  at 235 nm and  $\epsilon_{ETOH}^{269} = 23,000$  based on the  $^{14}C$  specific radioactivity. This spectrum is consistent only with a triene of the vitamin D type, but whether in the 5,6-*cis* form ( $\lambda_{max}$  265 nm,  $\epsilon^{265} = 18,200$ ) or as the 5,6-*trans* isomer ( $\lambda_{max}$  273.5 nm,  $\epsilon^{273.5} = 24,000$ ) is not clear. A hydroxyl group  $\alpha$  to the chromophore system could conceivably cause a small bathochromic or hypsochromic shift.

The distribution of  $^3H$  at C-1 in the [ $^3H$ ]-cholecalciferol preparation used in these studies was 85% at  $1\alpha$  and 15% at  $1\beta$  position<sup>11</sup>. The ratio of  $^3H/^{14}C$  in the isolated metabolite indicated a loss of at least 80% of the  $^3H$ , which is consistent only with the loss of the  $1\alpha$ -tritium. Such a loss is explicable only on the basis of a hydroxyl group at C-1, thereby eliminating the other possibilities in ring A, that is C-2 and C-4, that remain from the evidence of the mass spectra for the position of the third hydroxyl. Furthermore, the single peak at 269 nm in the ultraviolet spectrum eliminates the possibility of a hydroxyl at C-19 with loss of  $^3H$  by transposition of the double bond at C-10,19 to C-1,10. These data strongly suggest the structure

of the tritium-deficient metabolite of 25-hydroxycholecalciferol produced by the kidney as  $1\alpha,25$ -dihydroxycholecalciferol. It should be noted that as a result of inversion of ring A in the formation of vitamin D, the actual configuration at C-1 is the reverse of that indicated by the nomenclature, that is, the  $1\alpha$ -hydrogen is above the plane of the ring.

Whether 1,25-dihydroxycholecalciferol in the intestine remains as such or is further metabolized, as has been postulated to a ketone at C-1<sup>8,11</sup>, has to be established. Investigations into the metabolism of 1,25-dihydroxycholecalciferol to resolve this point are being carried out.

The biological activity of the kidney metabolite was tested in a preliminary experiment using the relative increase in the intestinal absorption of  $^{45}Ca$  as a criterion of activity. At a dose of 0.25  $\mu g$ , the 1,25-dihydroxycholecalciferol produced twice the degree of stimulation of  $^{45}Ca$  absorption, 16 h after administration to rachitic chicks, as did cholecalciferol. This compares with a three-fold increase in  $^{45}Ca$  absorption achieved with intestinal peak P compared with cholecalciferol.

DeLuca and his group have reported the identification of 21,25-dihydroxycholecalciferol<sup>12</sup> and 25,26-dihydroxycholecalciferol<sup>10</sup> isolated from pig plasma. These substances have variable vitamin D activity according to the method of assay, but are less active than 25-hydroxycholecalciferol. So far their presence has not been reported in tissues other than blood. Our previous results<sup>2,7</sup> with the double labelled vitamin suggest that they could account for only a minor proportion of the polar metabolites in bone and intestine.

The current view of the functional intermediary metabolism of cholecalciferol has been described recently<sup>6</sup>. The secretion by the kidney of small quantities of 1,25-dihydroxycholecalciferol and its accumulation by the target tissue means that this substance should properly be regarded as a hormone controlling calcium metabolism. The finite quantities of the metabolite in the tissues<sup>2</sup>, its characteristic accumulation in cell nuclei<sup>2,4</sup>, and the specific control over its synthesis<sup>6</sup> are necessary prerequisites for such a classification. In addition the effect of vitamin D resembles that of other steroid hormones in the changes it brings about in the template capacity of the DNA<sup>13</sup> and in RNA synthesis<sup>14-16</sup> in its target tissues.

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- Lawson, D. E. M., Wilson, P. W., and Kodicek, E., *Nature*, **222**, 171 (1969).
- Lawson, D. E. M., Wilson, P. W., and Kodicek, E., *Biochem. J.*, **115**, 269 (1969).
- Blunt, J. W., DeLuca, H. F., and Schnoes, H. K., *Biochemistry*, **7**, 3317 (1968).
- Haussler, M. R., Myrtle, J. F., and Norman, A. W., *J. Biol. Chem.*, **243**, 4055 (1968).
- Kodicek, E., Lawson, D. E. M., and Wilson, P. W., *Nature*, **228**, 763 (1970).
- Fraser, D. R., and Kodicek, E., *Nature*, **228**, 764 (1970).
- Lawson, D. E. M., Pelc, B., Bell, P. A., Wilson, P. W., and Kodicek, E., *Biochem. J.*, **121**, 673 (1971).
- Kodicek, E., in *The Fat-Soluble Vitamins*, Harry Steenbock Symp., Madison, Wisconsin, 1969 (edit. by DeLuca, H. F., and Suttie, J. W.) 81 (University of Wisconsin Press, 1970).
- Horsting, M., and DeLuca, H. F., *Biochem. Biophys. Res. Commun.*, **36**, 251 (1969).
- Suda, T., DeLuca, H. F., Schnoes, H. K., Tanaka, Y., and Hollick, M. F., *Biochemistry*, **9**, 4776 (1970).
- Bell, P. A., and Kodicek, E., *Biochem. J.*, **116**, 755 (1970).
- Suda, T., DeLuca, H. F., Schnoes, H. K., Ponchon, G., Tanaka, Y., and Hollick, M. F., *Biochemistry*, **9**, 2917 (1970).
- Hallick, R. B., and DeLuca, H. F., *Proc. US Nat. Acad. Sci.*, **63**, 528 (1969).
- Norman, A. W., *Biochem. Biophys. Res. Commun.*, **23**, 335 (1966).
- Stohs, S. J., Zull, J. E., and DeLuca, H. F., *Biochemistry*, **6**, 1304 (1967).
- Lawson, D. E. M., Wilson, P. W., Barker, D. C., and Kodicek, E., *Biochem. J.*, **115**, 263 (1969).

# Late DNA Replication in the Paternally Derived $X$ Chromosome of Female Kangaroos

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**The mode of dose compensation for  $X$  chromosomes in kangaroos seems to be inactivation of the paternal  $X$ , in contrast to the random  $X$  inactivation characteristic of eutherian mammals.**

FEMALE mammals have two  $X$  chromosomes, one inherited from each parent, while males have a single  $X$  chromosome inherited from the female parent only. Genes born on the  $X$  chromosome are thus carried in the hemizygous state in the male and their transmission to progeny is correlated with the sex of the parent. Seemingly homologous  $X$  linked genes are found in eleven species of eutherian mammals belonging to six different orders<sup>1</sup>. As a dose compensation mechanism one of the two  $X$  chromosomes carried by the female mammal is inactivated in somatic cells<sup>2</sup>. Numerous observations indicate that inactivation of maternally and paternally derived  $X$  chromosomes is random from cell to cell and that, once inactivated, the  $X$  remains inactive in all derivatives of the cell in which inactivation occurred. In female mice heterozygous for Cattanach's translocation of autosomal material into an  $X$  chromosome late DNA replication (late labelling) occurred in about equal numbers of maternally and paternally derived  $X$  chromosomes in several tissues<sup>3</sup>. In female kangaroos one of the two  $X$  chromosomes was always late in initiating and terminating DNA synthesis<sup>4</sup>. It has been suggested that the inheritance of glucose-6-phosphate dehydrogenase (G6PD) variation is  $X$  linked in marsupials but the patterns of inheritance suggest inactivation in the female of genes controlling G6PD production derived from the male parent<sup>5</sup>.

The  $X$  chromosome ( $X_e$ ) of euros (*Macropus robustus erubescens* Sclater), which have a slow moving electrophoretic form of G6PD<sup>5</sup>, is about one and a half times larger than the  $X$  chromosome ( $X_w$ ) of wallaroos (*Macropus r. robustus* Gould) which have fast moving G6PD. In other respects the karyotypes of the two subspecies are identical and interbreeding occurs readily in captivity. The female hybrid has  $X$  chromosomes of different sizes (Fig. 1). None of our hybrids have reached sexual maturity and so they cannot yet be tested for fertility although the same subspecies cross has elsewhere yielded fertile female offspring (personal communication from W. E. Poole). Euros and wallaroos ( $2n=16$ ) will cross with red kangaroos (*Megaleia rufa* (Desm.),  $2n=20$ ) yielding a sterile hybrid with eighteen chromosomes (Fig. 2). Two pairs of metacentric euro autosomes are composed of arms corresponding approximately in size to four smaller acrocentric red kangaroo autosomes and it has been suggested that the euro karyotype is derived from a twenty chromosome karyotype like that of the red kangaroo by the occurrence of two centric fusions<sup>6</sup>. The  $X$  chromosome of the red kangaroo ( $X_r$ ) is large by marsupial standards<sup>7</sup> and about twice the size of that of the euros ( $X_e$ ) so the sex chromosomes of male and female parents are readily distinguishable in the female hybrid.

In captivity, crosses were made between a female euro and a male wallaroo, a female wallaroo and a male red kangaroo

and a female red kangaroo and a male euro of Western Australian origin. Leucocyte cultures<sup>8</sup> were prepared from female offspring of all three crosses and the occurrence in these of sex chromosomes belonging to the respective male and female parents confirmed their hybrid origin. Thymidine (methyl T), specific activity 20.6 Ci/mmol (Amersham), was added to the cultures at a concentration of 0.25  $\mu$ Ci/ml.

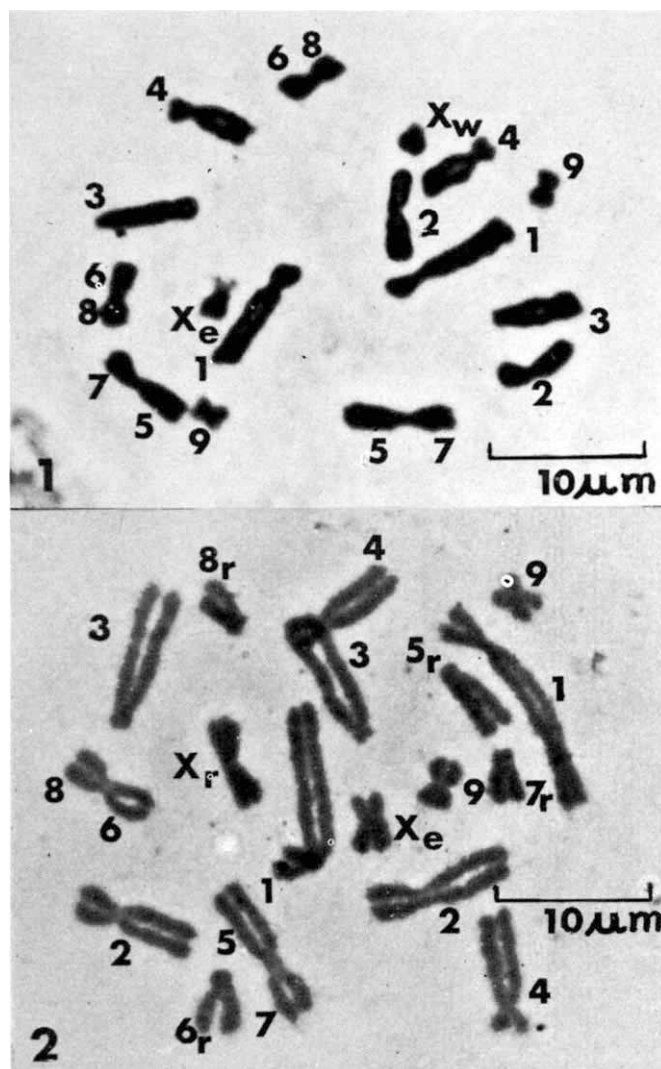


Fig. 1 The chromosomes of the female euro  $\times$  male wallaroo hybrid (SE31♀). The  $X$  chromosome derived from the female parent ( $X_e$ ) is larger than the  $X$  from the male parent ( $X_w$ ). Homologous chromosomes indicated by numbers. Autosomes with two numbers assumed to be derived from two ancestral acrocentric chromosomes (Fig. 2).

Fig. 2 The chromosomes of the female red kangaroo  $\times$  male euro hybrid (Q3♀). The  $X$  chromosome derived from the female parent ( $X_r$ ) is much larger than the  $X$  from the male parent ( $X_e$ ). Homologous chromosomes indicated by numbers. Known red kangaroo autosomes with suffix,  $r$ , the wallaroo metacentric autosomes assumed homologous with two red kangaroo acrocentrics with two numbers.



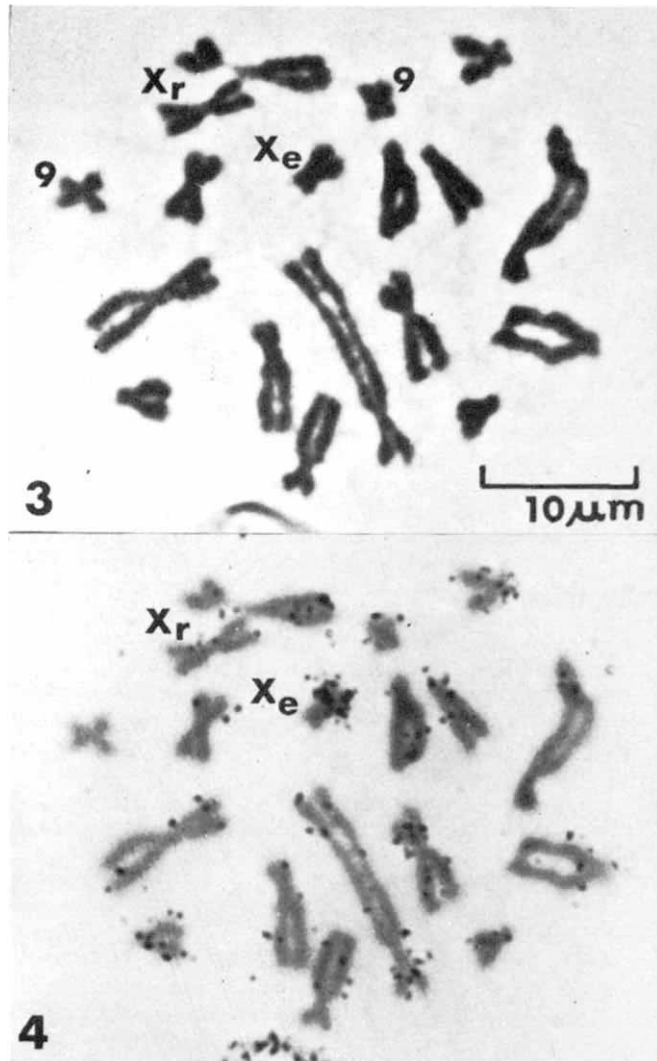


Fig. 3 The chromosomes of hybrid Q3♀ before addition of autoradiographic film. Labelling was as in Fig. 2.

Fig. 4 The same cell as in Fig. 3 after exposure to autoradiographic film. Long arm of paternally derived  $X$  chromosome ( $X_e$ ) positively labelled with respect to maternally derived  $X$  chromosome ( $X_r$ ) and to autosomes.

during the final hours of incubation. The maximum numbers of labelled metaphases were obtained from preparations to which the radioactive material was added 7 h before final collection. Colcemid (Ciba) at a final concentration of 0.025 mg/ml. was added to the cultures 2.5 h before collection. The  $X$  chromosomes of male and female origin were identified in metaphase cells in air dried preparations stained with aceto-orcin and the cells were photographed (Fig. 3) before addition of autoradiographic stripping film. The film was exposed to the preparations for 3–7 days before developing.

In preparations made from cultured cells of the euro  $\times$  wallaroo (SE31) and red kangaroo  $\times$  euro (Q3) hybrids the  $X$  chromosome of paternal origin showed almost universal late DNA replication compared with the  $X$  of maternal origin (Fig. 4). Tests for asynchrony of DNA replication between maternal and paternal  $X$  chromosomes and between the No. 9 chromosome pair (Figs. 1–3) were made in both hybrids by counting the number of grains on each pair. All counts in which one or more grains appeared on the chromosome pair were included in a homogeneity test in a  $2 \times N$  table, the test for agreement with a 1 : 1 ratio being a special case of the treatment suggested by Lewontin and Felsenstein<sup>9</sup>. Highly significant asynchrony of DNA synthesis occurred in the paternally derived  $X$  chromosomes of SE31 ( $\chi^2 = 79.046$ , 49 d.f.,  $0.01 > P > 0.001$ ) and Q3

( $\chi^2 = 76.942$ , 24 d.f.,  $P < 0.001$ ). No significant asynchrony was found between the No. 9 chromosomes of SE31 ( $P > 0.95$ ) or Q3 ( $0.5 > P > 0.2$ ). The asynchrony of DNA synthesis between marsupial  $X$  chromosomes, not separable as to maternal or paternal origin, shown by others<sup>4,10</sup> thus occurs in our material but the heterogeneity in this case is due to almost universal late replication of the  $X$  chromosome of paternal origin. In preparations from cultured cells of the wallaroo  $\times$  red kangaroo hybrid (BG2) the  $X$  chromosome of paternal origin (the larger of the two  $X$  chromosomes) always had a higher grain count than the smaller  $X$  of maternal origin.

In two of fifty cells in SE31 and two of twenty-five in Q3 the  $X$  chromosome of maternal origin had greater grain density and an excess of grains compared with the  $X$  of paternal origin. There was, however, no evidence for expression of the male G6PD locus in red blood cell precursors in the same hybrids<sup>5</sup>. Both  $X$  chromosomes of each hybrid had higher mean grain counts than the No. 9 autosomes of approximately equivalent size, so presumably each carries regions of genetic inactivity even when genetically activated at certain loci. Furthermore, the  $X$  chromosome of paternal origin was not always uniformly late labelling with respect to the alternate  $X$  or to the autosomes. In the cell illustrated in Fig. 4 the short arm of the paternal  $X$  ( $X_e$ ) is unlabelled and a like situation occurred in about 35% of cells analysed in each hybrid. This contrasts with the findings of Hayman and Martin<sup>7</sup>, who stated that the “smaller”  $X$  chromosomes of other marsupial species “behave uniformly over their length in the time of initiation and completion of their (DNA) synthesis”. In the female parent of SE31 late labelling of one of the equal sized small  $X$  chromosomes occurred as did non-uniform labelling. Hayman and Martin<sup>7</sup> reported non-uniform labelling in the  $X$  chromosomes of the red kangaroo which are “twice as big as the average smaller  $X$  chromosome group”. One of the female  $X$  chromosomes was uniformly late replicating while the other, and the single  $X$  of the male, had portions replicating at different times. How far these features of the parental  $X$  chromosomes affected the labelling patterns of the hybrid  $X$  chromosomes is unknown. An investigation of sex chromosome DNA replication and G6PD expression during ontogeny and reproductive cycles in the various tissues of marsupials is proceeding.

Late DNA replication of the  $X$  chromosome of paternal origin indicates at least partial genetic inactivation in most of the leucocytes. Other reports<sup>5,11</sup> show that in various kangaroo species  $X$  linked genes of the male parent are not expressed in somatic tissues of female offspring. The mode of dose compensation in kangaroos, and perhaps in all marsupials, thus seems to be paternal  $X$  inactivation in contrast to the random  $X$  inactivation characteristic of eutherian mammals.

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- <sup>1</sup> Ohno, S., *Ann. Rev. Genet.*, **3**, 495 (1969).
- <sup>2</sup> Lyon, M. F., in *Advances in Teratology* (edit. by Woolam, D. H. M.), **1** (Logos Press, London, 1966).
- <sup>3</sup> Evans, H. J., Ford, C. E., Lyon, M. F., and Gray, J., *Nature*, **206**, 900 (1965).
- <sup>4</sup> Graves, J. A. Marshall, *Exp. Cell Res.*, **46**, 37 (1967).
- <sup>5</sup> Richardson, B. J., Czuppon, A., and Sharman, G. B., *Nature New Biology* (in the press).
- <sup>6</sup> Martin, P. G., and Hayman, D. L., *Chromosoma*, **20**, 290 (1967).
- <sup>7</sup> Hayman, D. L., and Martin, P. G., in *Comparative Mammalian Cytogenetics* (edit. by Benirschke, K.) (Springer, New York, 1969).
- <sup>8</sup> Moorhead, P. S., Nowell, P. C., Mellman, W. J., Battips, D. M., and Hungerford, D. A., *Exp. Cell Res.*, **20**, 613 (1960).
- <sup>9</sup> Lewontin, R. C., and Felsenstein, J., *Biometrics*, **21**, 19 (1965).
- <sup>10</sup> Hayman, D. L., and Martin, P. G., *Cytogenetics*, **4**, 209 (1965).
- <sup>11</sup> Cooper, D. W., VandeBerg, J. L., Sharman, G. B., and Poole, W. E., *Nature New Biology* (in the press).

# LETTERS TO NATURE

## PHYSICAL SCIENCES

### Width of Cassini's Division in Saturn's Rings

It is commonly implied and sometimes explicitly stated in textbooks that the period of revolution corresponding to an orbit at the centre of Cassini's division in the rings of Saturn is half the period of Mimas, the closest massive satellite of Saturn, and that perturbations due to Mimas are responsible for the gap. In fact, the half period point of Mimas is 200 km beyond the outer edge of ring B rather than at the centre of the gap<sup>1</sup>. Moreover, the perturbations of Mimas are actually quite small—probably too small to account for the width of the gap, even if one disregards the mismatch between  $T_M/2$  and the revolution period at the centre of the gap. In fact, there is a simple way to obtain an approximate expression for the width of the gap produced in a ring of light particles by the perturbations of a massive body at the half-frequency point, assuming that both the massless particles in the ring and the outer body move in a central field produced by a very massive body.

We assume that the moderately massive body of mass  $m$  moves in a circular orbit of period  $T$  and of zero inclination about a much more massive centre of force with mass  $M$ . We expect that the perturbations due to  $m$  will cause a gap to appear in the ring material near  $T/2$ . From dimensional considerations one would expect that this gap (or region of low density of ring particles) should have a width given by

$$D = cR(m/M)^\alpha \quad (1)$$

Here  $D$  is the gap width,  $R$  is the radius of an orbit of period  $T/2$  and  $c$  and  $\alpha$  are constants. As we shall see,  $\alpha$  is in fact  $1/2$  and we will demonstrate this and calculate the constant  $c$  in a model. We consider a reference frame rotating with a particle in an initially circular orbit with period near (but not necessarily equal) to  $T/2$ . In this frame, the particle is at rest and the outer body revolves clockwise with period  $T$ . When the outer body actually has period  $T$  in this frame, the period of free motion of the inner particle under Coriolis and centrifugal forces is equal to the periodicity of the perturbing force due to the outer body. The result is a near-circular motion with steadily increasing radius (in this frame) of the inner particle, assumed initially at rest. This situation is particularly clear if we make some simplifications in the way we treat the problem.

Suppose that the perturbations of the outer body are concentrated in time at the point of closest approach to the inner particle and that the perturbations commence at some specific time. Then for this particular configuration, the orbit in the rotating frame is simple as long as its radius is small: the orbit is a circle in the rotating frame with one point fixed and with a steadily increasing radius. When the particle is not at a distance corresponding to a period  $T/2$  then, in the rotating frame, the outer body does not have a period equal to twice the period of free motion of the inner particle. Under the assumptions of the impulse approximation we have mentioned, the orbit of the inner particle is a circle which expands, rotates about a point on the circumference, finally contracts and then repeats

the process. This can lead to a net change in the semi-major axis of the orbit of the particle in the inertial frame.

If the outcome of this process is that the particle crosses the half-period point (where we expect the perturbations to build up rapidly), then we expect that the orbit would be disrupted. This gives a simple criterion for the width of the gap: if the circle expands and then contracts without causing the particle to cross the half-period point, we take the orbit to be satisfactorily stable. If this circle at its longest radius encompasses a segment of the half-period orbit, the particle orbit is unstable. Eventually a gap will be formed of half-width corresponding to the diameter of the first circle which is tangent to the "half-period" orbit.

It is a straightforward matter to arrange the phases of the successive perturbations so as to extract the criterion. Then the width of the gap becomes

$$\begin{aligned} D &= cR(m/M)^{1/2} \\ c &= (8\theta/3\pi F)^{1/2} \end{aligned} \quad (2)$$

where  $\theta$  is the angle over which the perturbing force is averaged in the impulse approximation and  $F$  is a function of the radius  $R$  from the centre of force to the lighter body and  $R'$  that to the massive body.

$$F = \left(\frac{R'}{R} - 1\right)^2 - \left[\left(\frac{R'}{R}\right)^{1/2} \cdot \left(1 - \frac{R}{R'}\right)^2\right] \quad (2a)$$

We have carried out a simplified numerical computation to check equation (2) by calculating the perturbations on a fixed circular orbit. These perturbations are very small for distances greater than a few times  $D$ . The effective angle to be used in (2) can be determined this way to be 1.42 radians.

Equation (2) can be checked by using it to calculate the gap width in the asteroid belt at the half-period point of the orbit of Jupiter<sup>2</sup>. With the value of  $\theta$  obtained from the computer calculation,  $D/R = 4.9\%$  compared to the actual gap width of between 4 and 5%, depending on the density criterion used to determine the size of the gap. We consider this to be satisfactory.

For the gap in the rings of Saturn, using equation (2), we find that Cassini's division ought to have a width  $D = 90$  km, with  $m/M = 6.7 \times 10^{-8}$ . The correct value is  $D = 3,000$  km, and for such a wide gap we would have to use a mass comparable to that of Titan in equation (2). Since the half-period point is not even at the centre of the gap but near to the outer edge of ring B, the perturbations due to Mimas are too small to produce a visible effect.

So what is responsible for Cassini's division? We suggest that the gap could have been created by some "proto-Mimas", which we visualize as a cloud of rock and gas significantly more massive than Mimas itself but less massive than Titan. Tidal forces alone would lead to a steady but small increase in the semi-major axis of the orbit of the body. The semi-major axis  $R$ , however, must have decreased by some distance of the order of 3,000 km to account for the location of the centre of the gap.

We tentatively assume that the mechanism for this decrease of the radius of the orbit is due to mass loss through the Lagrange points<sup>3</sup>  $L_1$  and  $L_2$ . These points can be quite close



to the body if  $R$  is fairly small. The result of the mass loss would be to deliver a small de-orbiting impulse to the cloud. We find

$$(R_f - R_i)/R_i = -c' (\omega_f/\omega_0)(m_i/M)^{2/3} \quad (3)$$

where  $R_i$  and  $R_f$  are the initial and final radii,  $m_i$  the initial mass ( $m \ll m_i$ ),  $\omega_0$  is the orbital angular frequency,  $\omega_s$  is a characteristic spin angular frequency for the cloud and  $c'$  is some positive constant the exact value of which depends strongly on the mechanism of mass loss. The principal variables are  $\omega_s/\omega_0$ , and  $m_i/M$ . If we suppose, to obtain an order of magnitude estimate, that  $(R_f - R_i)/R_i \approx -0.03$ , then for  $c' \approx 0.5$  and  $m_i \approx 0.1 M_{\text{Titan}}$ , we find  $\omega_s/\omega_0 \approx 66$ , a value we regard as not entirely unreasonable.

We believe that the problem of the division in the rings of Saturn may have interesting links with the early history of satellite systems, and in particular with the problem of the evolution of the satellites themselves.

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<sup>1</sup> Landolt-Börnstein, *Numerical Data and Functional Relationships in Science and Technology*, Group VI, 1, (Springer, Berlin, 1965).

<sup>2</sup> Message, P. J., in *The Theory of Orbits in the Solar System and Stellar Systems* (edit. by Contopoulos, G.), 197 (Academic Press, New York, 1966).

<sup>3</sup> Brandt, J. C., and Hodge, P. W., *Solar System Astrophysics*, 12 (McGraw-Hill, New York, 1964).

## Is Pluto an Iron-rich Planet?

Any plausible theory about the origin of the solar system must explain, among other known facts, the chemical fractionation of the planets. The terrestrial planets (of densities 3.9–5.5 g cm<sup>-3</sup>) are principally characterized by Fe, Mg, Si and oxygen, whereas some of the outer planets, of densities 0.7–1.6 g cm<sup>-3</sup> and rich in hydrogen, helium and other volatiles, may have retained the elemental composition of the original solar nebula. Hoyle and Wickramasinghe<sup>1,2</sup> have suggested that the planets were formed in a hot solar centrifuge<sup>3</sup>, and that whereas Jupiter and Saturn have the composition of the original solar nebula, the deficiency of hydrogen in Uranus and Neptune arises

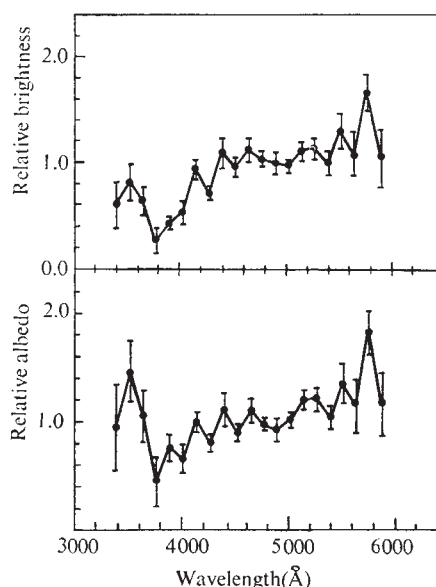


Fig. 1 Reflectance spectrum of Pluto, after Fix, Neff and Kelsey<sup>4</sup>.

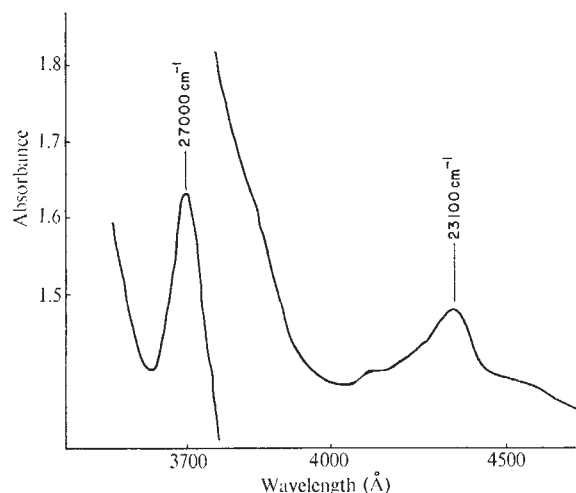


Fig. 2 Absorption spectra of terrestrial grossular garnet<sup>5</sup> at 293 K.

because the peripheral speed of hydrogen molecules is equal to the escape velocity from the solar system for these orbital distances. Further, the compositions of the more dense Fe-bearing inner planets may be caused essentially by thermal fractionation<sup>1–3</sup>, because refractory MgO, Fe and SiO<sub>2</sub> of the principal elements and possible molecules condense first at ~1500 K. The composition of Pluto is crucial to the validity of the nebular centrifuge theory, since from its position in the solar system the theory demands that Pluto not be Fe-rich. Here I show that similarities in the spectra of Pluto and Fe-bearing terrestrial silicates indicate that Pluto may well be Fe-rich.

The reflectance spectrum of Pluto reported by Fix, Neff and Kelsey<sup>4</sup> is shown in Fig. 1. In Fig. 2 I present the absorption spectrum of terrestrial grossular garnet<sup>5</sup>, showing the two prominent absorptions at 4330 Å and 3700 Å that mark the crystal-field transitions  ${}^6A_1 \rightarrow {}^4A_1 E(G)$  and  ${}^6A_1 \rightarrow {}^4E(D)$  in octahedral Fe<sup>3+</sup> ions, respectively. Two absorption bands at these approximate wavelengths are characteristic of spectra of Fe<sup>3+</sup>-bearing silicates<sup>6</sup>. Reflexion spectra of minerals are known to match the corresponding transmission or absorption spectra<sup>7</sup>. In addition, the reflectance spectrum of Mars<sup>8</sup> shows a decrease in reflectivity at 10000 Å that has been assigned to an absorption band marking the spin-allowed transition  ${}^2T_2(D) \rightarrow {}^2E(D)$  in ferrous iron. The most prominent feature in the Pluto spectrum at 3780 Å (Fig. 1) might therefore be considered to be an absorption band. Its half-width (~1,700 cm<sup>-1</sup>) compares favourably with the half-width of the 3700 Å (27,000 cm<sup>-1</sup>) grossular band (~1,000 cm<sup>-1</sup>), hence the Pluto band is likely to be of solid state origin. It seems reasonable to suggest further that the bands have a common origin, namely they mark the transition  ${}^6A_1 \rightarrow {}^4E(D)$  in Fe<sup>3+</sup> ions in oxide or silicate environments.

Although the assignment of a single band in a spectrum cannot be considered conclusive, there are other features in the Pluto spectrum that correlate well with known bands of Fe<sup>3+</sup>

Table 1 Wavelengths of Bands in Fe<sup>3+</sup> Spectra (Å)

|                            | Ion crystal | Andra-<br>dite <sup>6</sup> | Kimze-<br>yite <sup>8</sup> | YIG * <sup>10</sup> | Phlogo-<br>pite <sup>11</sup> | Pluto |
|----------------------------|-------------|-----------------------------|-----------------------------|---------------------|-------------------------------|-------|
| Octah. Fe <sup>3+</sup>    |             | 3,700                       |                             | 3,740               |                               | 3,780 |
|                            |             | 4,400                       | 4,400                       | 4,340               |                               | 4,300 |
| Tetrahed. Fe <sup>3+</sup> |             | 4,090                       | 4,120                       | 4,180               | 4,400                         | 4,020 |
|                            |             |                             | 4,860                       | 4,760               | 4,920                         | 4,900 |
|                            |             |                             |                             | 4,900               | 5,200                         |       |

\* Yttrium iron garnet.

**Table 2** Mean Densities ( $\text{g cm}^{-3}$ ) and Albedos of Planets<sup>19</sup>

| Object  | Mercury | Venus | Earth | Moon | Mars | Jupiter | Saturn | Uranus | Neptune | Pluto |
|---------|---------|-------|-------|------|------|---------|--------|--------|---------|-------|
| Density | 5.46    | 5.23  | 5.52  | 3.36 | 3.93 | 1.33    | 0.69   | 1.56   | 1.54    | ~4    |
| Albedo  | 0.06    | 0.76  | 0.36  | 0.07 | 0.16 | 0.73    | 0.76   | 0.93   | 0.62    | ~0.14 |

ions in minerals. For example, there seems to be a significant absorption at  $\sim 4300 \text{ \AA}$  ( $23,300 \text{ cm}^{-1}$ ) in the Pluto spectrum, which could mark the lowest field-independent transition<sup>5,6</sup>  ${}^6\text{A}_1 \rightarrow {}^4\text{A}_1\text{E}(\text{G})$  in octahedral  $\text{Fe}^{3+}$ . The energy separation of the  $4300 \text{ \AA}$  and  $3780 \text{ \AA}$  Pluto bands ( $\sim 3,300 \text{ cm}^{-1}$ ) compares favourably with the separations of the  ${}^4\text{A}_1\text{E}(\text{G})$  and  ${}^4\text{E}(\text{D})$  levels in  $\text{Fe}^{3+}$  ions in terrestrial silicates<sup>6</sup>. The Pluto spectrum also shows definite absorption at  $4900 \text{ \AA}$  ( $20,500 \text{ cm}^{-1}$ ) and possible absorption at  $4020 \text{ \AA}$  ( $24,900 \text{ cm}^{-1}$ ), which could mark the transitions<sup>9</sup>  ${}^6\text{A}_1 \rightarrow {}^4\text{T}_1(\text{G})$  or  ${}^4\text{T}_2(\text{G})$  and  ${}^4\text{A}_1\text{E}(\text{G})$  in tetrahedral  $\text{Fe}^{3+}$ . Band wavelengths are compared with those of the same transitions in  $\text{Fe}^{3+}$  in terrestrial crystals in Table 1.

Both  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$  ions exhibit a number of spin-forbidden bands in the visible region, and unless spectra of good resolution are available, it is often difficult assigning the bands<sup>12</sup>. Bands of  $\text{Fe}^{3+}$  in silicates, however, are usually more intense<sup>13,14</sup> than the visible-region bands of  $\text{Fe}^{2+}$ . There would seem to be sufficient correlations of energies and half-widths of bands in spectra of Pluto and terrestrial crystals to justify the suggestion that the surface of Pluto, at least, is iron-rich.

Although the physical properties of Pluto are not known accurately, the most probable values<sup>15-18</sup> of albedo ( $\sim 0.14$ ) and mean density ( $\sim 4 \text{ g cm}^{-3}$ ) support a model of an Fe-rich planet with only a very tenuous atmosphere. The albedo and density of Pluto are similar (Table 2) to those of Mars, which probably has an Fe-rich surface of oxide and silicate<sup>8</sup>. The Moon (albedo 0.07 and mean density  $3.36 \text{ g cm}^{-3}$ ) has a primarily basalt surface<sup>20</sup>. Allowing for inaccuracies in the determination of the mass of Pluto, the albedo and density are significantly different from those of the giant planets.

The presence of Fe on Pluto is inconsistent with the fractionation of refractory metals and their oxides as proposed by Hoyle<sup>1</sup> and Hoyle and Wickramasinghe<sup>2</sup>. If Pluto accreted in its present part of the solar system it would appear likely that all planets possess Fe-rich centres. On the other hand, it is possible that Pluto formed in an inner part of the solar system.

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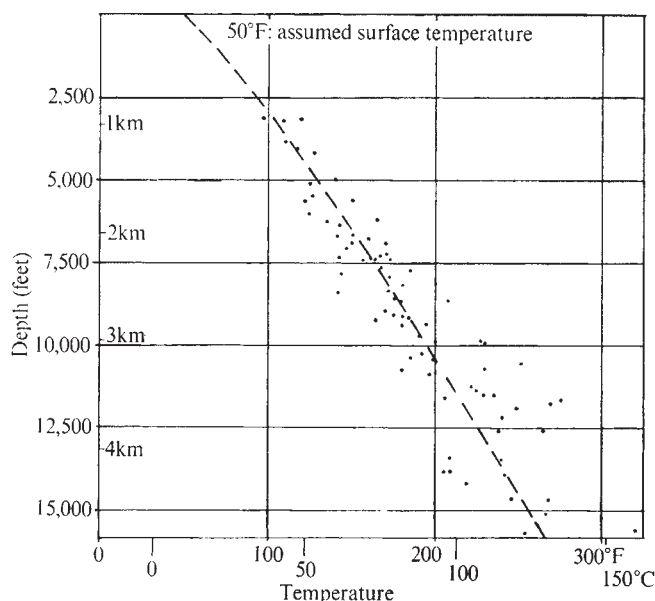
- <sup>1</sup> Hoyle, F., *Quart. J. Roy. Astron. Soc.*, **1**, 28 (1960).
- <sup>2</sup> Hoyle, F., and Wickramasinghe, N. C., *Nature*, **217**, 415 (1968).
- <sup>3</sup> Stubbs, P., *New Scientist*, **47**, 465 (1970).
- <sup>4</sup> Fix, J. D., Neff, J. S., and Kelsey, L. A., *Astron. J.*, **75**, 895 (1970).
- <sup>5</sup> Manning, P. G., *Canad. Mineral.*, **9**, 723 (1969).
- <sup>6</sup> Manning, P. G., *Canad. Mineral.*, **10**, 677 (1970).
- <sup>7</sup> Keester, K. L., and White, W. B., *Proc. Fifth Intern. Mineral. Assoc. Meeting.*, 22 (Cambridge, 1966).
- <sup>8</sup> Adams, J. B., *Science*, **159**, 1453 (1968).
- <sup>9</sup> Manning, P. G., *Nature*, **227**, 1121 (1970).
- <sup>10</sup> Wood, D. L., and Remeika, J. P., *J. Appl. Phys.*, **38**, 1038 (1967).
- <sup>11</sup> Faye, G. H., and Hogarth, D. D., *Canad. Mineral.*, **10**, 25 (1969).
- <sup>12</sup> Manning, P. G., *Nature*, **228**, 844 (1970).
- <sup>13</sup> Manning, P. G., *Canad. J. Earth Sci.*, **4**, 1039 (1967).
- <sup>14</sup> Manning, P. G., *Canad. Mineral.*, **9**, 237 (1967).
- <sup>15</sup> Kuiper, G. P., *Publ. Astron. Soc. Pacific*, **62**, 133 (1950).
- <sup>16</sup> Halliday, I., Hardie, R. H., Pranz, O. G., and Priser, J. B., *Astron. J.*, **70**, 676 (1965).
- <sup>17</sup> Halliday, I., *Publ. Astron. Soc. Pacific*, **81**, 285 (1969).
- <sup>18</sup> Kiladze, R., *J. Brit. Astron. Soc.*, **78**, 124 (1968).
- <sup>19</sup> *Observers Handbook*, Roy. Astron. Soc. Canad. (University of Toronto Press, 1971).
- <sup>20</sup> *Science*, **167**, 449 (1970).

## Approximate Geothermal Gradients in the North Sea Basin

APPROXIMATE geothermal gradients were determined from bottom hole temperatures for eighty wells in the North Sea Basin, which comprise about 30% of all post-1963 North Sea Basin wildcats. The wells are distributed as follows: onshore Netherlands, fifteen; offshore Netherlands, five; onshore Germany, one; offshore Germany, ten; offshore Denmark, one; offshore Norway, nine; offshore United Kingdom, thirty-nine. The data are displayed on the depth-temperature graph, Fig. 1, and the geothermal gradient contour map, Fig. 2. Before the determination of the geothermal gradients, an assumed surface temperature of  $10^\circ \text{C}$  was subtracted from the measured borehole temperature, and drilled depths were converted to depths below the sea bottom or land surface.

Temperatures, taken at hole bottom during electric log runs, were recorded 3-48 h after circulation of the drilling fluid was stopped. According to Lachenbruch and Brewer<sup>1</sup>, Jaeger<sup>2</sup> and Beck<sup>3</sup>, temperatures recorded in rotary-drilled holes soon after drilling operations have been completed are not equivalent to those of the undisturbed rock, because of the heat generated by the mechanical action of the bit on the rock face and the circulation and redistribution of heat by the mud system. I shall show in a later article, however, that geothermal gradients determined in this manner are likely to be in error by less than 10%.

In most North Sea wells several further temperature readings are available from intermediate log runs. A plot of these temperatures versus depths indicates in almost every case a decrease in geothermal gradient with depth. This decrease can also be observed by plotting the bottom hole geothermal gradient versus depth for all wells. The geothermal gradient in wells drilled in the deeper sub-basins of the North Sea basin (for example, the Tertiary basin centre near the Norwegian-



**Fig. 1** Depth-temperature relation for eighty wells in the North Sea Basin.



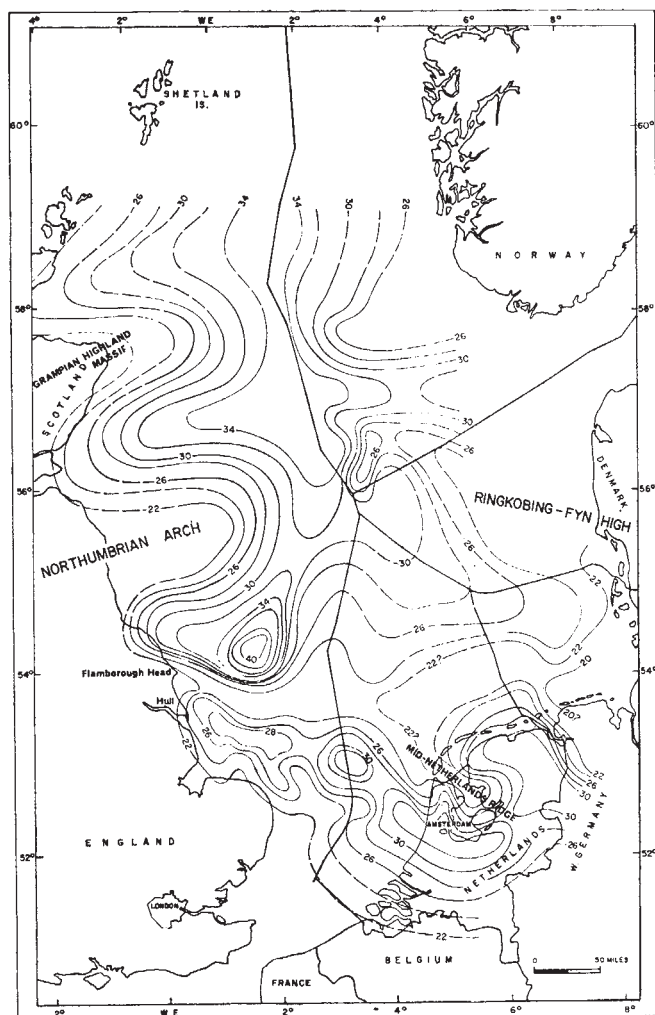


Fig. 2 Approximation of the geothermal gradient in the North Sea Basin based upon bottom hole temperatures recorded on mechanical well logs contours at  $2^{\circ}\text{C/km}$  interval.

United Kingdom median line, the Permian basin on the German and Dutch shelf and the Southwest Netherlands basin) decreases at an average rate of  $0.69^{\circ}\text{C/km}$  for the depth interval 7,000–16,000 feet. In the remainder of the North Sea the average rate of decrease is  $1.13^{\circ}\text{C/km}$  for the depth interval 4,000–12,000 feet. As a result of this decrease of gradient with depth, the geothermal gradient map (Fig. 2) is based on data which are not strictly comparable because of the varying total depths (3,200 to more than 15,000 feet).

The cause of the decrease in geothermal gradient with depth may be the increase in thermal conductivity with depth. An increase in this parameter for most rock types would accompany an increase in density. Such an increase in thermal conductivity would result in heating of the more thermally opaque overlying rocks.

The distribution of geothermal gradient highs and lows in the North Sea Basin is governed by the major structural elements; usually, low geothermal gradients occur over positive structural elements, and high geothermal gradients occur in deep post-Permian basins. The broad area with high geothermal gradients in the northern part of the North Sea coincides with the centre of the Tertiary sub-basin. This thermally positive trend probably results from the blanketing effect of thick Tertiary claystones and shales. A smaller (more areally restricted) positive thermal anomaly, with gradients locally exceeding  $40^{\circ}\text{C/km}$ , occurs east of Flamborough Head in the United Kingdom sector. The anomaly, which is indicated by three wells, does not coincide with any known structural or stratigraphic anomaly.

A negative thermal anomaly with an east-west trend extends from offshore Germany to south of Flamborough Head. This anomaly coincides approximately with the centre of the Permian evaporite sub-basin. Geothermal gradients derived from temperatures measured in the Permian and Carboniferous below the massive Permian salt beds are much lower than those derived from temperatures measured at the top of the salt beds. This suggests that the salt is an effective heat transfer agent, rapidly conducting the heat arriving near its base to the overlying rocks.

South of the negative thermal anomaly, a positive anomaly curves around the southern edge of the Permian salt basin from the North-East Netherlands to near Hull on the east coast of England. The anomaly coincides with the Southwest Netherlands basin over part of its length; thick Mesozoic rocks underlie the anomaly throughout its length.

This positive thermal anomaly is deeply embayed by a negative anomaly extending from the Permian sub-basin along the Mid-Netherlands Ridge. The low geothermal gradients measured in pre-Permian formations on the ridge are probably the result of shallow Carboniferous rocks with high thermal conductivities. Similar negative thermal anomalies occur over Hercynian or Caledonian highs extending into the North Sea Basin. These include the Northumbrian Arch, Ringkobing-Fyn High, and the Grampian Highland massif. Positive thermal anomalies extend into grabens or basins such as the Midland Valley Graben and the Moray Firth embayment between the positive structural elements.

To put the North Sea data into regional perspective, the average geothermal gradient and heat flow in Alpine basins<sup>4-6</sup> and Great Britain<sup>7</sup> are compared with these parameters in the North Sea Basin (Table 1). An average thermal conductivity of  $0.004$  to  $0.005\text{ calories cm}^{-1}\text{ s}^{-1}\text{ }^{\circ}\text{C}^{-1}$  is assumed for North Sea rocks.

Table 1 Average Geothermal Gradients and Estimated Heat Flow Values in Alpine Basins, Great Britain, and the North Sea Basin

|                               | Temperature gradient<br>$^{\circ}\text{C/km}$ | Average heat flow<br>$10^{-6}\text{ calories cm}^{-2}\text{ s}^{-1}$ |
|-------------------------------|-----------------------------------------------|----------------------------------------------------------------------|
| Bavarian Molasse Basin        | 28                                            | 1.35                                                                 |
| Carpatho-Ukraine Flysch Basin | $29 \pm$                                      | 1.05                                                                 |
| Transcarpathian Inner Deep    | $65 \pm$                                      | 2.6                                                                  |
| Vienna Basin                  | 25–35                                         | 1.2                                                                  |
| Little Hungarian Basin        | 45–50                                         | 2.0                                                                  |
| Great Britain                 | 32.3                                          | 1.31                                                                 |
| North Sea Basin               | 29.7                                          | 1.19–1.48                                                            |

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- Lachenbruch, A. H., and Brewer, M. C., *US Geol. Survey Bull.*, 1083C, 73 (1959).
- Jaeger, J. C., *Amer. Geophys. Union Geophys. Monog. Ser.*, No. 8, 7 (1965).
- Beck, A. E., *Amer. Geophys. Union Geophys. Monog. Ser.*, No. 8, 24 (1965).
- Boldizsar, T., *J. Geophys. Res.*, 73, No. 2, 613 (1968).
- Lubimova, E. A., *Isd. Nauka Moskva*, 50 (1966).
- Kul'chitskiy, D. I., and Osadchiy, V. G., *Akad. Nauk SSSR, Dokl.*, 179, No. 1, 152 (1968).
- Lee, W. H. K., and Uyeda, S., *Amer. Geophys. Union Geophys. Monog. Ser.*, No. 8, 87 (1965).

## Australia's Cenozoic Drift

A PROFOUND and continuing meridional movement of the Australian continent has been described by Carey<sup>1</sup> who pointed out that in the South Indian Ocean separating Australia and Antarctica (Fig. 1), the opposing coasts are parallel and that the intermediate median rise is the locus of much seismic activity. The direction of the dilational movement is recorded, Carey believed, by the roughly meridional oceanic lineament connecting Tasmania and Victorialand, and the date of the beginning of rifting by the Jurassic dolerites of these areas. He said also that the pole path determined by Irving<sup>2</sup> from a palaeomagnetic study of Australian Mesozoic and Cenozoic rocks had roughly the same length and trend as this lineament.

New data and inferences from morphological and geomagnetic studies confirm Carey's concept in outline and can provide the framework for a coherent interpretation of Australia's Cenozoic history. Precise morphological analyses<sup>3,4</sup> show that the assembly obtained by moving Australia and Antarctica together along the Tasmania-Victorialand lineament is the best fit, and rivals that of Africa and South America in its precision. Mapping of magnetic lineaments on the South Indian Ocean floor reveals a pattern, extending from continent to continent, that is bilaterally symmetrical about the median Indian-Antarctic Ridge<sup>5</sup>, which is believed to record a Cenozoic separation of the continents commencing about 45 m.y. ago. Tertiary palaeomagnetic data for Australia<sup>6</sup> indicate a northerly drift of Australia of about 15° of latitude since that time which equals the present distance between the southern margin of Australia and the axis of the Indian-Antarctic Ridge.

Thus it can be inferred from morphological and geomagnetic evidence that at the beginning of the Cenozoic, the South Indian Ocean did not exist and Australia was joined to Antarctica. Commencing about 45 m.y. ago (mid-Eocene) Australia and Antarctica began to drift apart, Australia moving north 15° to reach its present position. I intend to examine this inference and to assess its validity in its ability to explain some of the gross aspects of continental geology.

Analysis of the disintegration of Gondwanaland led Smith and Hallam<sup>4</sup> to conclude that "much of the initial rifting probably occurred in Jurassic and Lower Cretaceous times, but much of the dispersal occurred in Upper Cretaceous and Tertiary times". This generalization certainly seems valid for the separation of Australia and Antarctica. Carey<sup>7</sup> envisages the evolution of the southern margin of the Australian continent thus: "initial Upper Jurassic rifting produced a Red Sea-like trough between Australia and Antarctica on the site of the Great Australian Bight, which (still like the Red Sea) forked into a Bassian rift valley between Tasmania and Victoria (c.f. Gulf of Suez) and another rift valley between Antarctica and western Tasmania (c.f. Gulf of Aquaba) isolating Tasmania (which is about the size and shape of Sinai). This configuration persisted throughout the Cretaceous and the Paleocene, and it was only during the Tertiary that the large separation occurred."

The carbonate sedimentation characteristic of Australia's southern margin<sup>8</sup> seems to have commenced with a late Eocene marine transgression which ended paralic sedimentation initiated in the late Jurassic or early Cretaceous<sup>9-12</sup>. (The advance of this transgression within the Otway Basin of western Bass Strait, culminating in late Oligocene/early Miocene time, is documented in ref. 12.) The onset of this transgression, which marked the length of Australia's southern margin, seems to coincide with, or closely follow, the onset of Austral-Antarctic dispersal as dated by geomagnetism, suggesting a genetic relationship. Seismic investigations of the continental shelf, west of Bass Strait (offshore Otway Basin), have revealed the presence near the shelf edge of a seaward dipping unconformity between paralic sediments of the late Cretaceous and abyssal marine sediments of the early Oligocene age. The characteristics of this unconformity are consistent



Fig. 1 Morphology of the southern ocean and bordering continents. Continents, delineated by 2,000 m depth contour, unshaded. Ocean floor shaded: dark tone, 2,000 m to 4,000 m; light tone, >4,000 m. BS, Bass Strait; T, Tasmania; V, Victorialand; LHR, Lord Howe Rise; NC, New Caledonia; OS, Owen Stanley. After Carey<sup>1</sup>.

with large scale seaward slumping in the late Eocene or early Oligocene<sup>13</sup> and may reflect the gravitational instability which might be anticipated along a newly formed continental margin.

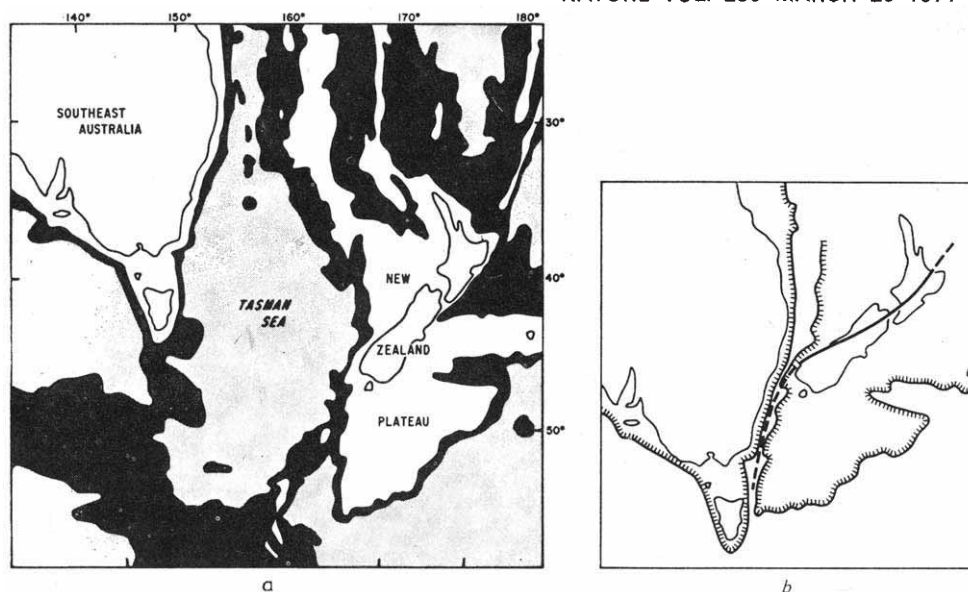
A terminal Eocene event no less significant than the late Eocene event which affected Australia's southern margin is indicated by the stratigraphy of the northern margin of the Australian continent in New Guinea. At the beginning of the Cenozoic, the northern margin of the Australian continent seems to have coincided with New Guinea's mountainous spine<sup>14,15</sup>. While dominant shoal carbonates accumulated on the continental shelf, pillow lavas, radiolarites and pelagic limestones were accumulating in the oceanic environment to the north. At the close of the Eocene, this late Mesozoic-early Cenozoic continental margin became the site of intense tectonism. In the Oligocene the oceanic edge of the continental shelf emerged and began to shed sediment to the south, reversing the direction of Mesozoic provenance and establishing a tectonic-sedimentary regime which continues to the present<sup>14,15</sup>. Rocks of the adjacent seafloor were also involved in the emergence of the Eocene continental margin, detritus from them first appearing in early Miocene sediments. In eastern New Guinea post-Eocene tectonism was accompanied by andesitic volcanism which persists to the present<sup>15</sup>, associated with strong seismic activity<sup>16</sup>.

Australia's Cenozoic drift has profound implications for a perennial problem of south-west Pacific geology—the relationship between Australia and New Zealand and the origin of the Tasman Sea. If Australia's Cenozoic drift is reversed, then it is apparent that a southerly translation of 15° along the Tasmania-Victorialand lineament is sufficient to close the Tasman (Figs. 1 and 2). Furthermore, this motion highlights morphological congruence between the Tasman continental margins of Australia and the New Zealand Plateau (Fig. 2).

If the dispersal of Australia and New Zealand and the creation of the Tasman Sea is a consequence of Australia's Cenozoic drift, then the onset of dispersal must have occurred in or after the late Eocene. What evidence is there of the date of this event?



Fig. 2 *a*, Morphology of the Tasman Sea and bordering continents. Shadings as in Fig. 1. After Connolly<sup>20</sup>. *b*, Reassembly of Australia and New Zealand Plateau. Continuous to broken line, Alpine Fault and postulated former extension.



The south-eastern margin of mainland Australia, from Cape Howe (lat. 37° S) to Sandy Cape (lat. 25° S), truncates a NNW continental grain in rocks ranging from Ordovician to Cretaceous in age. The youngest rocks truncated, which presumably set a maximum age limit to the onset of drift, are folded strata of mid-Cretaceous (Albian) age in the Maryborough Basin<sup>9</sup>. A minimum age limit for the same region is set by the early Miocene limestone which unconformably overlies probable Cretaceous rocks in a well on the Great Barrier Reef, 80 km seaward of the onshore Maryborough Basin<sup>17</sup>.

The only portion of the south-eastern margin of the Australian continent with a sedimentary record spanning the crucial early Tertiary interval is the Gippsland Basin of eastern Bass Strait. Probably initiated in the late Jurassic as the eastern portion of a "Bassian Rift Valley"<sup>7</sup>, the Gippsland Basin accumulated a great thickness of predominantly non-marine sediments during the late Jurassic, Cretaceous and early Tertiary<sup>11,18</sup> and as along the southern margin of the continent, early Tertiary fluvial-deltaic sediments are succeeded by dominant marine carbonates after a late Eocene transgression<sup>11,19</sup>.

If Connolly's<sup>20</sup> interpretation of well and seismic data is valid, submarine canyons like those which now mark the continental slope of east Bass Strait first came into existence in the late Eocene or early Oligocene. Accurate dating of the onset of canyon development and other continental margin-related phenomena, which can be expected from current intensive investigations associated with oil search in the Gippsland Basin, will provide the surest indication of the onset of Tasman dilation.

Apart from its morphology, little is known of the eastern margin of the oceanic Tasman Sea—the western margin of the New Zealand Plateau. Most of New Zealand, the emergent portion of the mostly submerged plateau, lies a considerable distance from the continental margin and might be expected to yield little data on its evolution. Yet the early Tertiary stratigraphic record of New Zealand's western or Tasman coast is similar to that of the Gippsland Basin and Australia's southern margin. Here too a late Eocene marine transgression, progressing from south-west to north-east or outwards from the Tasman margin of the New Zealand Plateau, ended the accumulation of coal measures and initiated marine sedimentation which by Oligocene times was predominantly calcareous<sup>9,21</sup>.

I think that the small amount of data available favours a late Eocene age for the onset of Tasman dilation. Several authors who have recently touched on the subject follow Carey<sup>1</sup> in assuming that the New Zealand Plateau attained its present gross structural configuration after the onset of Tasman

dilation. In their reconstructions the effects of the Alpine Fault are eliminated before New Zealand is joined to Australia in a reconstituted form. Such reconstructions imply that the dispersal of Australia and New Zealand commenced before the late Jurassic–early Cretaceous interval in which the principal movement on the Alpine Fault took place<sup>22</sup>. Moreover, if once contiguous margins can be shown to be preserved undeformed, as I suggest, then Tasman dilation must have occurred after the New Zealand Plateau assumed its present gross structural configuration in the late Mesozoic, a condition which a late Eocene date fulfils.

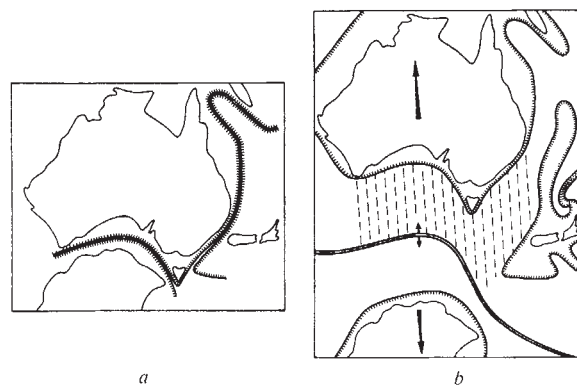


Fig. 3 Postulated Cenozoic dispersal of Australia and adjacent continents. *a*, Pre-drift configuration; *b*, present configuration. Dashed line (*b*) marks path of Australia relative to New Zealand Plateau and mid-ocean ridge. Fig. 1 was used as the basis for this.

In spite of the bathymetric, geotectonic and Cenozoic stratigraphic data in its favour, this Australia/New Zealand reassembly is not without problems. It lacks any unequivocal geological and palaeogeographic continuity, and in particular New Zealand's Alpine Fault with its 450 km of transcurrent displacement seems to have no extension into the Australian mainland. Both aspects of this continuity problem may be largely resolved if the southern extension of the Alpine Fault once formed the great fracture along which the continents were subsequently to part (Fig. 2*b*). In the postulated reassembly the Alpine Fault meets the Australian continent at the northern end of an abrupt continental slope, slightly concave in plan, which extends at present from lat. 33° S to lat. 38° S<sup>23</sup>. This

margin, and its New Zealand counterpart, may record the former southern course of the Alpine Fault.

A final problem which Australia's Cenozoic drift may solve is the origin of the Coral Sea. South-east of the Coral Sea lie New Caledonia and Chesterfield Reef, rising from the northern extremities of the Norfolk Ridge and Lord Howe Rise. As continental extensions of the New Zealand Plateau, each must find a place in an Australia/New Zealand reconstruction. It has been recognized<sup>24</sup> that New Caledonia forms part of a south-west Pacific stratigraphic belt linking New Zealand's Northland peninsula to the Owen Stanley peninsula of New Guinea. The physical continuity of this belt is disrupted by the oceanic crust of the Coral Sea<sup>25</sup>, the line of displacement between these landmasses, which may once have been adjoining, being of comparable length and trend to that inherent in the postulated Tasman dilation. It can be readily envisaged that at the onset of Australia's Cenozoic drift the northern portion of the Lord Howe Rise and New Caledonia Ridge lay within the Coral Sea Basin, in physical continuity with the Australian continent along what is now its Coral Sea margin (Fig. 3a). As Australia drifted north, these entities, like the New Zealand Plateau to which they appear to be joined, were left behind (Fig. 3b).

Data relevant to the time of origin of the Coral Sea are still more sparse than for the Tasman. A recent geophysical investigation of the Coral Sea suggests that the deeply submerged but apparently continental Queensland Plateau subsided to its present position during the late Tertiary<sup>25</sup>, perhaps as a result of collapse along a continental margin newly created by the withdrawal of the Lord Howe Rise.

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- <sup>1</sup> Carey, S. W., *Continental Drift—a Symposium*, 177 (Univ. Tasmania, 1958).
- <sup>2</sup> Irving, E., *Continental Drift—a Symposium*, 177 (Univ. Tasmania, 1958).
- <sup>3</sup> Sproll, W. P., and Dietz, R. S., *Nature*, **222**, 346 (1969).
- <sup>4</sup> Smith, A. G., and Hallam, A., *Nature*, **225**, 139 (1970).
- <sup>5</sup> Le Pichon, X., and Heirtzler, J. R., *J. Geophys. Res.*, **73**, 2101 (1968).
- <sup>6</sup> Wellman, P., McElhinny, M. W., and McDougall, I., *Geophys. J. Roy. Astron. Soc.*, **18**, 371 (1969).
- <sup>7</sup> Carey, S. W., *Advances in the Study of the Sydney Basin*, 53 (Univ. Newcastle, 1969).
- <sup>8</sup> Connolly, J. R., and von der Borch, C. C., *Sediment. Geol.*, **1**, 181 (1967); Wass, R. E., Connolly, J. R., and Macintyre, R. J., *Marine Geol.*, **9**, 63 (1970).
- <sup>9</sup> Brown, D. A., Campbell, K. S. W., and Crook, K. A. W., *The Geological Evolution of Australia and New Zealand* (Pergamon, Oxford, 1968).
- <sup>10</sup> Connolly, J. R., Flavell, A., and Dietz, R. S., *Marine Geol.*, **8**, 31 (1970).
- <sup>11</sup> Reynolds, M. A., *J. Austral. Petrol. Explor. Assoc.*, 50 (1967).
- <sup>12</sup> Taylor, D. J., *Geol. Surveys Victoria and S. Aust. Paper*, 10 (in the press).
- <sup>13</sup> Taylor, D. J., *Completion Report Esso Nautilus-1 Well* (unpublished but available under Comm. Austral. Subsidy Acts).
- <sup>14</sup> Hermes, J. J., *Geol. en Mijnbouw*, **47**, 81 (1968).
- <sup>15</sup> Thompson, J. E., *J. Austral. Petrol. Explor. Assoc.*, 83 (1967).
- <sup>16</sup> Denham, D., *J. Geophys. Res.*, **74**, 4290 (1969).
- <sup>17</sup> Derrington, S. S., Crespini, I., Morgan, W. R., Bock, P. E., and Houston, B. R., *Publ. Petrol. Search Subsidy Acts Austral.*, 4 (1960).
- <sup>18</sup> Traill, T. R., *J. Austral. Petrol. Explor. Assoc.*, 67 (1968).
- <sup>19</sup> Hocking, J. B., and Taylor, D. J., *J. Austral. Petrol. Explor. Assoc.*, 125 (1964).
- <sup>20</sup> Connolly, J. R., *Marine Geol.*, **6**, 439 (1968).
- <sup>21</sup> Sprigg, R. C., Braithwaite, J. C., Yakunin, A., and Wilson, R. B., *Amer. Assoc. Petrol. Geol. Bull.*, **34**, 856 (1969).
- <sup>22</sup> Suggate, R. P., *Trans. Roy. Soc. N.Z. (Geology)*, **2**, 105 (1963); Fleming, C. A., *Quart. J. Geol. Soc. Lond.*, **125**, 125 (1969).
- <sup>23</sup> Connolly, J. R., *N.Z. J. Geol. Geophys.*, **12**, 310 (1969).
- <sup>24</sup> Glaessner, M. F., *Amer. Assoc. Petrol. Geol. Bull.*, **34**, 856 (1950).
- <sup>25</sup> Ewing, M., Hawkins, L. V., and Ludwig, W. J., *J. Geophys. Res.*, **75**, 1953 (1970).

## Seismotectonics of the Australian Continent

ONE of the successes of the seafloor spreading hypothesis has been the coherent explanation it provides of world seismicity. Isacks *et al.*<sup>1</sup> have given an account of seismic activity along mid-ocean ridges and island arcs in accordance with the concepts of plate tectonics, and McKenzie<sup>2</sup> has shown that these concepts can be applied also to instantaneous motions across continental plate boundaries. It seems reasonable, therefore, to attempt an analysis of continental seismicity within plates in terms of the same hypothesis. Morgan<sup>3</sup> and Le Pichon<sup>4</sup> have shown that the assumption of perfectly rigid plates gives results which are in approximate accord with observation. Nevertheless, their results are unlikely to be adversely affected—and may well be improved—if inhomogeneous stresses within plates are postulated to explain patterns of earthquake activity in continental regions.

Because of the relative infrequency of earthquakes in continental areas removed from plate boundaries, many years of monitoring by regional networks are usually required before specific patterns of seismicity emerge. Epicentral data for the Australian continent from the beginning of the century until the end of 1966 have been tabulated and discussed by Doyle *et al.*<sup>5</sup>. The quantity and quality of data improved considerably from about 1959, corresponding to increases in the number of Australian stations, and since 1966 the addition of more stations in central and western Australia has further improved the seismic monitoring of these regions.

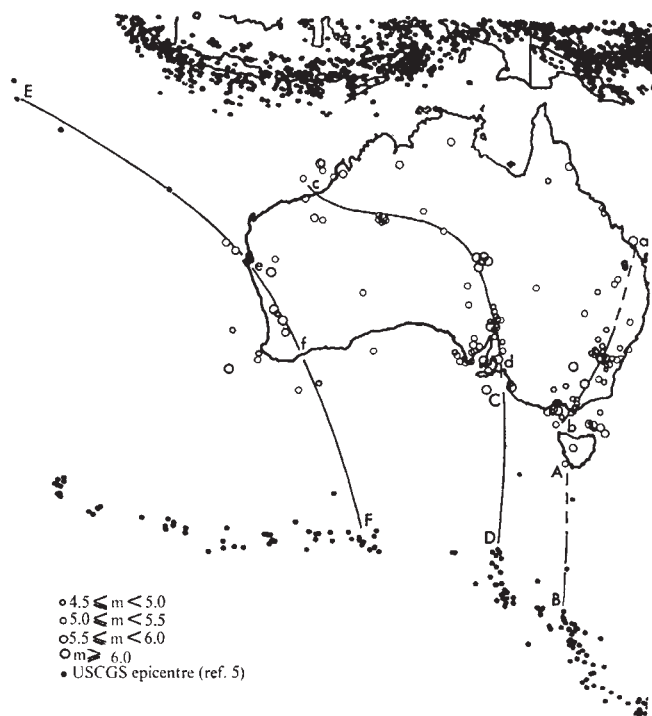


Fig. 1 Australian seismicity in relation to that of the Antarctic ridge and the Indonesian-New Guinea arc. The lettering is explained in the text.

In Fig. 1 the seismicity map of Doyle *et al.*<sup>5</sup>, with the more recent epicentres added, has been superimposed on a portion of the ESSA 1961–1969 world seismicity map<sup>6</sup> showing events in the surrounding oceanic areas. Sykes<sup>7</sup> considers that the stepped shape of the ridge-fracture zone south of Tasmania reflects the shape of the south-east Australian continental slope. The orientations of transform faults in this zone also indicate a northward migration of Australia relative to the Antarctic plate, in approximate agreement with the differential movements calculated by Le Pichon<sup>4</sup>.



Fig. 1 shows three major Australian seismic zones. In south-east Australia, the zone *a-b* probably represents a late stage of the post-middle Miocene Kosciusko uplift described by David<sup>8</sup>. From fault-plane studies, Cleary<sup>9</sup> and Underwood<sup>10</sup> have inferred a horizontal, principal compressive stress acting at right angles to the strike of *a-b*, with high-angle reverse movements typical of the larger shocks. In the middle of the continent, the apparently continuous zone *c-d* coincides with a feature postulated by Cook<sup>11</sup>, Wilson<sup>12</sup> and Crawford<sup>13</sup> to be an ancient fracture zone. In the south-west corner is a zone *e-f*, first described by Everingham<sup>14</sup>, within which large earthquakes have recently occurred at Meckering<sup>15</sup> and elsewhere.

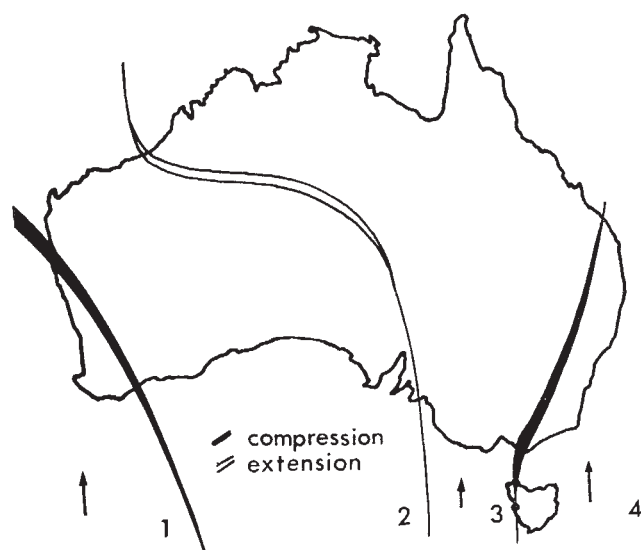


Fig. 2 Division of Australia into four sub-plates. Differences in spreading rate are represented schematically by the lengths of the arrows.

Possible extensions of these zones beyond the continental shelf have also been indicated in Fig. 1. *A-B* and *C-D* are oceanic fracture zones depicted by Heirtzler *et al.*<sup>16</sup>. Wilson<sup>17</sup> has postulated that transform faults originate on lines of weakness within continents, and the coincidence of continental and oceanic fracture zones in these regions strongly suggests the operation of such a mechanism. The presence of epicentres close to these features may be indicative of minor seismic activity, difficult to detect by the existing network. Sykes<sup>7</sup> has suggested that the Indian Ocean epicentres along the line *E-F* may represent a nascent stage in the development of an island arc. Doyle also believes that this and other aspects of Australian seismicity may be due to second order effects within the plate. (Paper delivered at intern. conf. on recent crustal movements, Wellington, New Zealand, 1970.)

We now postulate that differences in spreading rate, indicated by discontinuities in seismic activity along the Antarctic ridge, are accompanied by the production of stress gradients within the plate, which are in turn responsible for the patterns of seismicity already described. In view of this, the section of the ridge marked *F-D* is interesting. Within *F-D*, which is about 1,000 km long, almost no seismic activity has been detected since Gutenberg and Richter's original study of world seismicity<sup>18</sup>. There is evidence also of a break in the seismicity of the ridge at *B*, with a change in activity west of this point. No corresponding variations in activity can be recognized along the arc to the north of Australia; it is not known, however, how much of this activity is caused by relative motion of the south-east Asian plate.

In Fig. 2, the continent has been divided into four sub-plates. From the lack of seismicity along *F-D*, we infer that the

spreading rate of sub-plate 2 is small compared with the others. The spreading rates of 1, 3 and 4 relative to 2 are schematically indicated in the figure. The model takes account of the observed features of Australian seismicity, and predicts that the strains in north-west Australia are extensional, as distinct from those in the south-west and south-east regions. The configuration shown here is not necessarily stable over long periods. During the main phase of the Kosciusko uplift, for example, the motion of sub-plate 4 relative to 3 may have been greater than at present. Earlier cycles of uplift could perhaps be similarly explained.

Within the framework I have outlined, it becomes possible to account more specifically for the tectonic behaviour of these seismic regions in terms of the properties of the upper mantle low velocity layer. Hales *et al.*<sup>19</sup> have shown that observed differences in the travel times of seismic body waves are closely related to regional variations in the low velocity layer, possibly due to differences in the extent of partial melting. The P wave station anomalies listed by Cleary and Hales<sup>20</sup> have high positive values in areas of recent tectonic uplift, low positive values at oceanic stations, and negative values in shield areas. These results imply that the low velocity zone is best developed beneath tectonically active areas and least developed under shields. A similar conclusion has been reached by Kanamori<sup>21</sup>, based on a study of Love and Rayleigh wave phase velocities. Cleary<sup>22</sup> has confirmed that P wave station anomalies in Australia follow this trend. In particular, stations in south-east Australia have large positive anomalies, indicating a strongly developed low velocity layer and probably, therefore, a substantial zone of low strength in the upper mantle asthenosphere.

Presumably, portions of the asthenosphere which are intrinsic to particular tectonic provinces are carried along by the moving lithosphere, otherwise it is difficult to explain how such differences persist. In these circumstances, it is conceivable that the "soft" layer beneath south-east Australia (*a-b*) is being compressed by the more rigid upper mantles of the shield and oceanic regions on either side, resulting in uplift of the crustal block. In shield areas, on the other hand, the less strongly developed low velocity layer is less susceptible to deformation. Thus in the south-west Australian shield regions (*e-f*) no significant uplift has occurred and the fault motion may be predominantly strike-slip, while in the shield area of north-west Australia (*c-d*) the applied stresses are most easily relieved by rifting along ancient zones of weakness.

The casual sequence underlying the foregoing model remains undetermined. Are inactive sections of the oceanic ridge the cause of variations in the stress field within the plate? Are both phenomena the effects of collision between India and Asia? Or alternatively, are they the result of variations in viscous drag beneath the continent, produced by lateral inhomogeneities in the asthenosphere? Whatever the relationship, it seems certain that discontinuities in the production of lithosphere along the ocean ridge must be accompanied by inhomogeneous stresses which extend inwards from the plate margin. These have been linked, in our model, with the observed patterns of seismicity within Australia; we are hopeful that the concept will assist the study of seismicity of other continents.

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<sup>1</sup> Isacks, B., Oliver, J., and Sykes, L. R., *J. Geophys. Res.*, **73**, 5855 (1968).

<sup>2</sup> McKenzie, D. P., *Nature*, **226**, 239 (1970).

<sup>3</sup> Morgan, W. J., *J. Geophys. Res.*, **73**, 1959 (1968).

<sup>4</sup> Le Pichon, X., *J. Geophys. Res.*, **73**, 3661 (1968).

<sup>5</sup> Doyle, H. A., Everingham, I. B., and Sutton, D. J., *J. Geol. Soc. Austral.*, **15**, 295 (1968).

- <sup>6</sup> U.S. Department of Commerce, ESSA, *World Seismicity, 1961-1969*, Map NEIC-3005 (1970).
- <sup>7</sup> Sykes, L. R., *J. Geophys. Res.*, **75**, 5041 (1970).
- <sup>8</sup> David, T. W. E. (edit. by Browne, W. R.), *The Geology of the Commonwealth of Australia* (Arnold, London, 1950).
- <sup>9</sup> Cleary, J. R., thesis, Australian National Univ. (1963).
- <sup>10</sup> Underwood, R., thesis, Australian National Univ. (1967).
- <sup>11</sup> Cook, P. J., *Rec. Bur. Min. Res. Geol. Geophys. Austral.*, **46** (1966).
- <sup>12</sup> Wilson, J. T., *Science, History and Hudson Bay* (edit. by Beals, C. S.), 2 (Queen's Printer, Ottawa, 1968).
- <sup>13</sup> Crawford, A. R., *Econ. Geol.*, **65**, 11 (1970).
- <sup>14</sup> Everingham, I. B., *Rec. Bur. Min. Res. Geol. Geophys. Austral.*, **132** (1968).
- <sup>15</sup> Everingham, I. B., Gregson, P. J., and Doyle, H. A., *Nature*, **223**, 701 (1969).
- <sup>16</sup> Heitzler, J. R., Dickson, G. O., Herron, E. M., Pitman, W. C., III, and Le Pichon, X., *J. Geophys. Res.*, **73**, 2119 (1968).
- <sup>17</sup> Wilson, J. T., *Nature*, **207**, 343 (1965).
- <sup>18</sup> Gutenberg, B., and Richter, C. F., *Seismicity of the Earth*, first ed. (Princeton Univ. Press, 1949).
- <sup>19</sup> Hales, A. L., Cleary, J. R., Doyle, H. A., Green, R., and Roberts, J., *J. Geophys. Res.*, **73**, 3885 (1968).
- <sup>20</sup> Cleary, J. R., and Hales, A. L., *Bull. Seism. Soc. Amer.*, **56**, 467 (1966).
- <sup>21</sup> Kanamori, H., *Phys. Earth Planet. Interiors*, **2**, 259 (1970).
- <sup>22</sup> Cleary, J. R., *Bull. Seism. Soc. Amer.*, **57**, 773 (1967).

## New Method of Collagen Extraction for Radiocarbon Dating

DATING by the radiocarbon method is of primary importance for archaeological studies and on many sites bones are the only samples which can be dated<sup>1</sup>. The losses arising from the destruction of bones for dating purposes is not very important, whereas it is a pity to destroy clothes or wooden items associated with the civilization being studied. It is also more logical to use bones for dating an archaeological level than to use wood or artefacts, for example, which are not necessarily contemporary with the site occupation<sup>2</sup>. There are, however, frequent and often important errors in <sup>14</sup>C bone measurements which arise chiefly because of the difficulty of eliminating completely the numerous pollutants during chemical treatment.

Several methods of preparation have been proposed (for example, refs. 2-5), but they have the disadvantages that some contaminants remain after the treatment, or, if they are completely eliminated, the treatment is too severe and most of the original collagen is also lost.

We describe in this article a method of obtaining a good yield of pure collagen which depends on the solubility of collagen in slightly acidic hot water after acid pretreatment of bones; a very pure gelatine can be obtained in this way<sup>6</sup>.

An 8% HCl solution is used to eliminate most mineral substances from the crushed bones and also some organic pollutants. The acid also breaks some of the hydrogen bonds of collagen, so that it becomes soluble in hot water. The

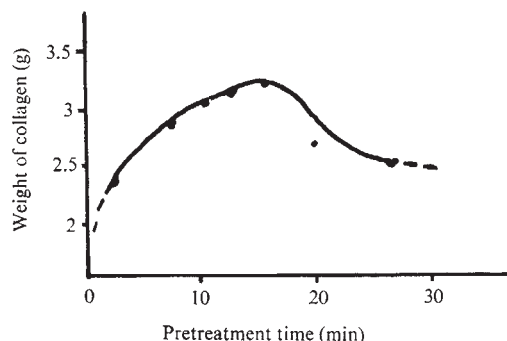


Fig. 1 Dependence of the weight of collagen extracted from 50 g of bones on the pretreatment time with 8% HCl.

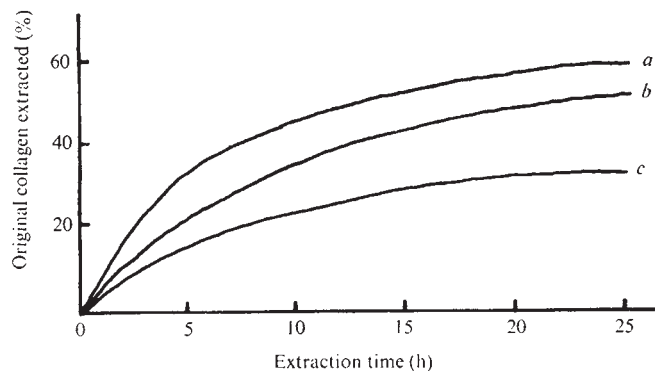


Fig. 2 Extraction yield of collagen from fossil bones in hot acidic water (90°C, pH=3.0). a, 12 min acid pretreatment; b, 20 min acid pretreatment; c, 30 min acid pretreatment.

pretreatment time depends on the fineness of the bone powder but must be short otherwise the proteinic chain is hydrolysed and the collagen becomes soluble in cold water and is then lost (Fig. 1).

The collagen is extracted by heating the residue with water (pH=3) at about 90°C and stirring for many hours. Only collagen is present in the gelatine formed; the impurities remain in the residue and can be eliminated by centrifugation. The pure gelatine can be burnt after drying in an oven to form the gas sample for counting purposes. The quantity of bone to be treated is determined by a Kjeldahl dosage. The maximum yield is about 65 to 70% and depends especially on the hot water dissolution time; 50% of the original collagen can be extracted after 10 h if the pretreatment has been short (Fig. 2).

Table 1 Experimental Conditions for producing Usable Quantities of Collagen Gelatine

|                     | Crushing diameter | Acidity                            | Temperature (°C) | Time   |
|---------------------|-------------------|------------------------------------|------------------|--------|
| Acid pre-treatment  | 0.2 mm            | 1 l. 8% HCl<br>(for 100 g of bone) | 20               | 20 min |
| Extraction in water |                   | pH=3.0                             | 90               | 10 h   |

The experimental conditions for producing sufficient gelatine from fossil bones are shown in Table 1. During the acid pretreatment the carbonates are eliminated as well as the organic contaminants soluble in acid and after the collagen has dissolved in hot water, the roots, foreign bodies (saprophytes, woods and so on) and other organic pollutants (such as humic acids which are almost insoluble in the acid medium) remain in the residue.

Bone samples were chosen from different types of archaeological site (rock shelter or open air site, calcareous or sandy deposits) to check the degree of impurity elimination and thus the validity of <sup>14</sup>C dating by this method. In every case unburned bones were associated with charcoals or burned bones on which the classical preparation method<sup>7</sup> has been performed (HCl and N NaOH).

A comparison of the results is shown in Table 2; there is close agreement between the dates obtained for the bones and those for the charcoals or burned bones. The measurements also agree well with the archaeologically expected dates. Two of the three dates for humic fraction of charcoals (extract) are younger and prove the elimination of pollutants in collagen preparation (see also refs. 6 and 7).

Apart from the thorough elimination of pollutants, this method has the advantages of rapidity of preparation (2 h of attentive work), simple operation, simple chemical reagents and good yield. It can be used for partially burned bones<sup>6</sup>, and probably for the extraction of the conciline of shells,



**Table 2** Comparison Between  $^{14}\text{C}$  Dates of Collagen from Fossil Bones and from Associated Charcoals or Burned Bones

| Site                   | Sample No.     | Expected age<br>(yr)          | $^{14}\text{C}$ age                |                                            |                  |
|------------------------|----------------|-------------------------------|------------------------------------|--------------------------------------------|------------------|
|                        |                |                               | From collagen<br>of unburned bones | From charcoals or burned bones<br>Reliquat | Extract          |
| La Couronne-Martigues  | Ly 301/302     | 4,000–4,300                   | 3,970 $\pm$ 130                    | 4,060 $\pm$ 220                            | —                |
| Montclus               | Ly 303/304     | Late Cardial 6,000–6,500      | 6,140 $\pm$ 140                    | 6,300 $\pm$ 140                            | —                |
| Montclus Layer 21      | Ly 305/306     | Sauveterrian 7,500–8,300      | 7,780 $\pm$ 250                    | 7,890 $\pm$ 170                            | —                |
| Montclus Layer 22      | Ly 307/308     | C14 Köln lab. 8,130 $\pm$ 240 | 7,750 $\pm$ 340                    | 7,770 $\pm$ 410                            | —                |
| St-Remèze              | Ly 318/319/320 | Azilian (~11,000)             | 11,500 $\pm$ 380                   | 11,750 $\pm$ 300                           | 12,080 $\pm$ 310 |
| Les Deux-Avens         | Ly 321/322     | 12,000                        | 12,350 $\pm$ 200                   | 12,320 $\pm$ 600                           | —                |
| Solutré                | Ly 314/315/316 | Middle Solutrean (~19,000)    | 17,150 $\pm$ 300                   | 16,740 $\pm$ 300                           | 10,900 $\pm$ 400 |
| St-Martin-ss-Montaignu | Ly 309/310/311 | Upper Perigordian (~25,000)   | 22,900 $\pm$ 600                   | 24,150 $\pm$ 550                           | 21,100 $\pm$ 130 |

which has the same physico-chemical property as I have described for collagen. The pure gelatine can be dissolved in a solvent and introduced directly into a liquid scintillation counter.

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- <sup>1</sup> Tammers, M. A., and Pearson, F. J., *Nature*, **208**, 1053 (1965).
- <sup>2</sup> Sellstedt, H., Engstrand, L., and Getjwall, N. G., *Nature*, **212**, 572 (1966).
- <sup>3</sup> Haynes, C. V., *IAEA Symp. Dating and Method of Low Level Counting*, Monaco, 163 (1967).
- <sup>4</sup> Berger, R., Horney, A. G., and Libby, W. F., *Science*, **144**, 999 (1964).
- <sup>5</sup> Kruegger, H. W., Sixth Intern. Conf. C14 and T3 dating, 332 (Pullmann, Washington, 1965).
- <sup>6</sup> Longin, R., thesis, Univ. Lyon (1970).
- <sup>7</sup> Evin, J., Longin, R., Marien, G., and Pachiaudi, Ch., *Radiocarbon*, **13**, No. 1 (1971).

fission tracks. Once formed, the fission tracks disappear if the material is heated above a critical temperature. The fission track age,  $T$  (yr), can be represented by the following equation<sup>3</sup>

$$T = \frac{1}{\lambda} \ln \left[ 1 + \frac{\lambda}{\lambda_f} \frac{\rho_s}{\rho_i} \frac{\Phi \sigma}{\eta} \frac{R_i}{R_s} \right] \quad (1)$$

where  $\rho_s$  is the fossil fission track density ( $\text{cm}^{-2}$ ),  $\rho_i$  is the induced track density by bombardment with thermal neutrons ( $\text{cm}^{-2}$ ),  $\lambda$  is the total decay constant for uranium ( $\text{yr}^{-1}$ ),  $\lambda_f$  is the fission decay constant for  $^{238}\text{U}$  (we use  $6.85 \times 10^{-17} \text{ yr}^{-1}$  (ref. 4)),  $\Phi$  is the thermal neutron dose ( $\text{cm}^{-2}$ ),  $\sigma$  is the thermal neutron cross section for fission  $^{235}\text{U}$  ( $\text{cm}^{-2}$ ),  $\eta$  is the isotope ratio  $^{235}\text{U}/^{238}\text{U}$ , and  $R_s$  and  $R_i$  are the mean values of the etchable length of the fragments produced by a spontaneous fission and that produced by the induced fission (cm).

If  $T$  were smaller than  $10^9$  yr and  $R_s \approx R_i$ , equation (1) can be written

$$T = 6.12 \times 10^{-8} \Phi \frac{\rho_s}{\rho_i} \quad (2)$$

The uranium content of minerals and glasses was obtained by induced fission in a reactor.

We have studied three groups of materials: (1) slags, "Tatara", provided by Professor S. Wajima of the University of Okayama; (2) minerals in baked earths, pottery and tiles; and (3) a glass ball and glaze on a bowl.

Zircons were separated from the baked relics using the standard separating methods: heavy liquids and isodynamic separator. The glasses were mounted in a clear epoxy resin on a polymer slide and the mount was then ground and

## Fission Track Dating of Archaeological Materials from Japan

THE method of dating by fission tracks<sup>1</sup> has been used by several workers to date rock minerals and baked relics<sup>2</sup>. The method depends on the spontaneous fission of  $^{238}\text{U}$  atoms in mineral or glass taking place at a constant rate, and leaving

**Table 1** Fission Track Ages of Archaeological Materials from Japan

| Sample                    | Locality                              | $s$ ( $\text{cm}^{-2}$ ) | $i$ ( $\text{cm}^{-2}$ ) | ( $\text{cm}^{-2}$ )  | Fission track age (yr BP) |
|---------------------------|---------------------------------------|--------------------------|--------------------------|-----------------------|---------------------------|
| Zircon (baked earth)      | Ise, Hata, Mie Pref.                  | $4.7 \times 10^3$        | $5.7 \times 10^7$        | $0.46 \times 10^{15}$ | 2,300                     |
| Zircon (baked earth)      | Hirakata, Osaka Pref.                 | $2.0 \times 10^3$        | $2.5 \times 10^7$        | $0.46 \times 10^{15}$ | 2,200                     |
| Zircon (baked earth)      | Hisai, Mie Pref.                      | $1.0 \times 10^4$        | $1.8 \times 10^8$        | $0.44 \times 10^{15}$ | 1,500                     |
| Glass ball                | Hirono, Uji, Kyoto Pref.              | $6.3 \times 10^2$        | $4.8 \times 10^5$        | $2.31 \times 10^{15}$ | 1,480                     |
| "Tatara"                  | Ichinomiya, Okayama City              | $3.1 \times 10^2$        | $4.1 \times 10^7$        | $2.81 \times 10^{15}$ | 1,330                     |
| Zircon (baked earth)      | Iwasaki, Nisshin, Aichi Pref.         | $3.6 \times 10^4$        | $7.5 \times 10^8$        | $0.44 \times 10^{15}$ | 1,300                     |
| Zircon (baked earth)      | Sakai City                            | $2.7 \times 10^3$        | $6.3 \times 10^7$        | $0.46 \times 10^{15}$ | 1,220                     |
| Zircon (tile)             | Nagaoka, Kyoto Pref.                  | $8.3 \times 10^3$        | $2.0 \times 10^8$        | $0.50 \times 10^{15}$ | 1,250                     |
|                           |                                       | $8.3 \times 10^3$        | $2.1 \times 10^8$        | $0.50 \times 10^{15}$ | 1,200                     |
|                           |                                       | $7.7 \times 10^3$        | $2.0 \times 10^8$        | $0.50 \times 10^{15}$ | 1,200                     |
|                           |                                       | $7.0 \times 10^3$        | $1.9 \times 10^8$        | $0.50 \times 10^{15}$ | 1,150                     |
| Zircon (pottery)          | Nagaoka, Kyoto Pref.                  | $7.0 \times 10^3$        | $1.9 \times 10^8$        | $0.50 \times 10^{15}$ | 1,150                     |
| "Tatara"                  | Habutenno, Wake, Okayama Pref.        | $2.2 \times 10^2$        | $1.7 \times 10^7$        | $1.47 \times 10^{15}$ | 1,150                     |
| Zircon (opening of folge) | Arai, Fukaya, Shiraishi City          | $6.8 \times 10^3$        | $1.8 \times 10^8$        | $0.50 \times 10^{15}$ | 1,150                     |
| "Tatara"                  | Michikunihara, Fukaya, Shiraishi City | $2.2 \times 10^2$        | $3.3 \times 10^7$        | $2.81 \times 10^{15}$ | 1,160                     |
|                           |                                       | $2.1 \times 10^2$        | $3.4 \times 10^7$        | $2.80 \times 10^{15}$ | 1,090                     |
|                           |                                       | $2.0 \times 10^2$        | $3.3 \times 10^7$        | $2.82 \times 10^{15}$ | 1,060                     |
| "Tatara"                  | Habutenno, Wake, Okayama Pref.        | $1.5 \times 10^2$        | $2.4 \times 10^7$        | $2.81 \times 10^{15}$ | 1,050                     |
|                           |                                       | $1.6 \times 10^2$        | $2.4 \times 10^7$        | $2.80 \times 10^{15}$ | 1,150                     |
|                           |                                       | $1.5 \times 10^2$        | $2.4 \times 10^7$        | $2.82 \times 10^{15}$ | 1,100                     |
| "Tatara"                  | Odatemori, Ajigazawa, Aomori Pref.    | $7.8 \times 10$          | $9.8 \times 10^6$        | $1.47 \times 10^{15}$ | 720                       |
| Zircon (baked earth)      | Fuso, Aichi Pref.                     | $2.3 \times 10^4$        | $8.8 \times 10^8$        | $0.44 \times 10^{15}$ | 700                       |
| Glaze on bowl             | Isumi, Toki City                      | $4.5 \times 10$          | $1.1 \times 10^5$        | $1.9 \times 10^{15}$  | 400                       |

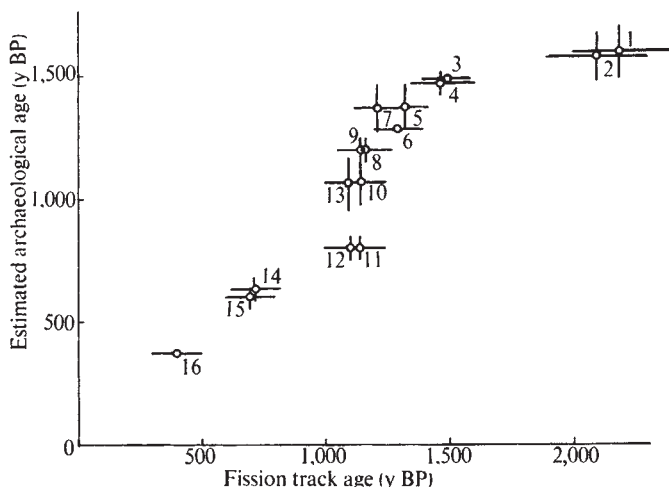


Fig. 1 The relationships between archaeological age and fission track age of some Japanese relics.

polished to expose the interior of the glasses. Zircon required from 1–2 min in concentrated  $P_2O_5$  at  $450^\circ$ – $500^\circ$  C or 1–4 h in 10 M NaOH at  $220^\circ$  C. The glass, on the other hand, was etched in 46% HF at room temperature; this etchant required 2–40 s to etch tracks in the glass. After the sample had been etched and washed, it was ready for counting.

For the reactor run, a small amount of zircon and glass was packed in a plastic container and irradiated in a reactor. Neutron flux was also obtained by the fission track method<sup>5</sup>. For example, details of the zircon from the baked earth collected from Hisai, Mie Prefecture, is given as follows:

$$T = 6.12 \times 10^{-8} \times 0.44 \times 10^{15} \times \frac{1.01 \times 10^4}{1.82 \times 10^8} = 1,500 \text{ yr BP}$$

The results of the experiments are given in Tables 1 and 2. As shown in Fig. 1, the results of the fission track method agree with archaeologically estimated ages, but several questions still remain unsolved.

Table 2 Uranium Content of some Relics

| Sample and source |                              | U (p.p.m.) |
|-------------------|------------------------------|------------|
| Glaze on a bowl,  | Hm-1 (Tajimi, Hime-Gama-1)   | 2.5        |
|                   | Kr-78 (Kariya, Kurozasa-78)  | 3.0        |
|                   | Kr-35 (Miyoshi, Kurozasa-35) | 3.0        |
|                   | Kr-35 (Miyoshi, Kurozasa-35) | 3.2        |
|                   | N-1 (Ena, Nagata)            | 3.0        |
|                   | Ko-1 (Seto, Kochoso)         | 3.9        |
|                   | Ko-2 (Seto, Kochoso)         | 3.7        |
|                   | S-1 (Nagoya, Higashiyama-11) | 3.6        |
|                   | M-1 (Toki, Motoyashiki)      | 3.5        |
|                   | M-1 (Toki, Motoyashiki)      | 3.2        |
| Glass ball (1)    | (Bozuyama-1, Uji)            | 21.2       |
|                   | (2)                          | 1.8        |
|                   | (Bozuyama-1, Uji)            | 1.8        |

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<sup>1</sup> Price, P. B., and Walker, R. M., *J. Geophys. Res.*, **68**, 4847 (1963).

<sup>2</sup> Watanabe, N., and Suzuki, M., *Nature*, **222**, 1057 (1969).

<sup>3</sup> Fleischer, R. L., and Price, P. B., *Geochim. Cosmochim. Acta*, **28**, 1705 (1964).

<sup>4</sup> Fleischer, R. L., and Price, P. B., *Phys. Rev.*, **133B**, 63 (1964).

<sup>5</sup> Hashimoto, T., Iwata, S., Nishimura, S., Nakanishi, T., and Sakanoue, M., *Proc. Ninth Jap. Conf. Radioisotopes*, 231 (1969).

## BIOLOGICAL SCIENCES

### Identification of Prostaglandin $F_{2\alpha}$ in the Utero-ovarian Blood of Guinea-pig after Treatment with Oestrogen

THE relative importance of the pituitary gland and the uterus in controlling the lifespan of the corpus luteum in mammals has been the subject of extensive investigation. In some species, the local nature of the effect of the uterus on the corpora lutea seems to preclude the involvement of the pituitary gland in luteolysis; while on the other hand the effect of gonadal steroids on luteal regression has usually been attributed to an action at the pituitary level<sup>1</sup>. The fact that the luteolytic action of oestrogen is abolished in the hysterectomized guinea-pig<sup>2</sup>, however, reaffirms the importance of the uterus.

During studies aimed at the identification of the uterine luteolytic factor it has been shown that prostaglandin  $F_{2\alpha}$  ( $PGF_{2\alpha}$ ) can induce luteolysis in the rat<sup>3</sup>, rabbit<sup>4</sup>, guinea-pig<sup>5</sup>, sheep<sup>6</sup> and monkey<sup>7</sup>. The fact that distension of the guinea-pig uterus *in vivo* hastens luteal regression<sup>8</sup>, and  $PGF_{2\alpha}$  is released *in vitro* by this means<sup>9</sup>, strongly suggests that  $PGF_{2\alpha}$  may be the physiological luteolysin. Accordingly, utero-ovarian venous blood has been collected from guinea-pigs in which secretion of the luteolytic factor was enhanced by prior treatment with oestrogen, and the content of  $PGF_{2\alpha}$  determined.

Guinea-pigs were injected subcutaneously with 10  $\mu$ g of oestradiol benzoate/day from days 4–6 of the cycle (day 1 = day of oestrus). On day 7, each was anaesthetized with pentobarbitone sodium and injected with 5,000 IU of heparin intravenously. The utero-ovarian vein on one side was exposed, but because of the frailty of the vein and its readiness to collapse, no attempt was made to separate it from the artery running alongside, nor were any side branches ligatured. Blood from the vein was collected into a cooled tube by means of a needle and silastic catheter for approximately 1–1.5 h. Centrifugation at  $4^\circ$  C was carried out as soon after collection as possible and the plasma was stored at  $-20^\circ$  C until extracted. Five guinea-pigs were hysterectomized on day 4, treated with 10  $\mu$ g of oestradiol benzoate/day from days 4–6, and blood collected from the intact utero-ovarian vein on day 7. Blood from untreated guinea-pigs was similarly collected for control purposes.

Plasma from four or five guinea-pigs was pooled in each experiment to give approximately 30 ml. Plasma from untreated guinea-pigs was also pooled. Prostaglandins were extracted by solvent partition and partially purified by silicic acid column chromatography<sup>10</sup>. Biological assay of the eluted fractions on the rat fundal strip detected prostaglandin F-like activity in the plasma extracts from intact guinea-pigs treated with oestrogens but not in the controls, nor in the oestrogen treated hysterectomized group (Table 1). None of the samples contained any prostaglandin E-like activity.

The methyl ester-trimethyl silyl ether derivative of the extracted material was prepared. Gas chromatography gave a peak at the retention time corresponding to this derivative of authentic prostaglandin  $F_{2\alpha}$ . Mass spectra of the unknowns showed all the principal *m/e* peaks of authentic prostaglandin  $F_{2\alpha}$ . Plasma from control and from oestrogen-treated hysterectomized guinea-pigs contained no detectable prostaglandin  $F_{2\alpha}$  on gas chromatography or when mass spectra were taken on the gas chromatographic column effluent at the appropriate retention time<sup>11</sup>.

In six out of eight experiments biological assay showed more prostaglandin  $F_{2\alpha}$  in the intact animals pretreated with oestrogen than in controls. In two of these experiments the identification and quantification were confirmed by combined gas chromatography-mass spectrometry.

The conclusive demonstration of an endogenous secretion of  $PGF_{2\alpha}$ , a substance known to be luteolytic, into the utero-ovarian vein of oestrogen-treated guinea-pigs is of great



significance. That this secretion seems to be favoured by the steroid satisfactorily accounts for the luteolytic action of oestrogen in the intact guinea-pig, which is abolished in the hysterectomized animal. It also seems likely that a similar mechanism could be involved in the normal cycle, with  $\text{PGF}_{2\alpha}$  secretion increasing under the influence of oestrogen from the growing follicles at about day 10 of the cycle.

**Table 1** Concentration (ng/ml.) of Prostaglandin  $\text{F}_{2\alpha}$  in Blood taken from the Utero-ovarian Vein on Day 7 of the Oestrous Cycle of Oestrogen-treated and Control Guinea-pigs

| Experiment No. | Oestrogen-treated | Untreated |
|----------------|-------------------|-----------|
| 1              | 19–25             | <3        |
| 2              | 25–29             | <3.8      |
| 3              | 11–13             | <6.7      |
| 4              | 3.3               | —         |
| 5              | 2.2–4.2           | <5.2      |
| 6              | 9–11†             | <2.2      |
| 7              | 4.2–6.2           | <1.9      |
| 8              | 11–13†            | <1.9*     |

\* Hysterectomized oestrogen-treated <1.8.

† Identification confirmed by gas chromatography-mass spectrometry.

Although this work supports the view that  $\text{PGF}_{2\alpha}$  is involved in luteolysis and establishes a means by which secretion is initiated, the mechanism of action of  $\text{PGF}_{2\alpha}$  in the luteolytic process is still unknown.

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- Anderson, L. L., Bland, K. P., and Melampy, R. M., in *Rec. Prog. Hormone Res.* (edit. by Astwood, E. B.), **25**, 57 (Academic Press, London and New York, 1969).
- Bland, K. P., and Donovan, B. T., *J. Endocrinol.*, **47**, 225 (1970).
- Pharriss, B. B., and Wyngarden, L. J., *Proc. Soc. Exp. Biol. Med.*, **130**, 92 (1969).
- Gutknecht, G. D., Cornette, J. C., and Pharriss, B. B., *Biol. Reprod.*, **1**, 367 (1969).
- Blatchley, F. R., and Donovan, B. T., *Nature*, **221**, 1065 (1969).
- McCracken, J. A., Glew, M. E., and Scaramuzzi, R. J., *J. Clin. Endocrinol. Metab.*, **30**, 544 (1970).
- Kirton, K. T., Pharriss, B. B., and Forbes, A. D., *Proc. Soc. Exp. Biol. Med.*, **133**, 314 (1970).
- Bland, K. P., and Donovan, B. T., *J. Physiol.*, **186**, 503 (1966).
- Poyser, N. L., Horton, E. W., Thompson, C. J., and Los, M., *Nature* (in the press).
- Samuelsson, B., *J. Biol. Chem.*, **238**, 3229 (1963).
- Thompson, C. J., Los, M., and Horton, E. W., *Life Sci.*, **9**, 983 (1970).

## Reversal of Pargyline-induced Inhibition of Sexual Behaviour in Male Rats by *p*-Chlorophenylalanine

SIDE effects of monoamine oxidase inhibitors (MAOI) include frigidity and impotence in male patients<sup>1,2</sup>. Meyerson has shown that brain serotonin inhibits hormone-induced oestrous behaviour in castrated female rats<sup>3–6</sup> and other studies have indicated that brain serotonin inhibits sexual behaviour in male rats<sup>7–9</sup>, rabbits<sup>10</sup> and cats<sup>11</sup>. We have therefore investigated the relation of reduced sexual sensitivity produced by pargyline, an MAOI, to accumulation of brain serotonin, and whether the effect could be prevented by *p*-chlorophenylalanine (PCPA), a potent inhibitor of serotonin biosynthesis<sup>12</sup>. The effect of PCPA alone on the sexual behaviour of male animals with receptive females was also studied.

Male Sprague-Dawley rats, 140–150 days old at the beginning of the experimental period, were housed individually in observation cages from 1 week before any treatment began. The lighting schedule consisted of 14 h of light (0600 h to 2000 h) and 10 h of darkness. The mating tests were done from 2200 h–2400 h in a dim light. For each test a female in oestrus was introduced in each observation cage with the male. The female rats used in this study were Sprague-Dawley rats ovariectomized 3 weeks before use. They were brought into heat by subcutaneous injections of oestradiol and progesterone in olive oil<sup>5</sup>.

The following parameters were scored: (a) the percentage of animals copulating at least once within 30 min after the rats were put together; (b) the percentage of animals ejaculating at least once within 60 min after the rats were put together; (c) the mean number of ejaculations before the animals reached our criterion of exhaustion: that is, when more than 30 min elapsed between one ejaculation and the next.

**Table 1** Effect of Pargyline, PCPA and the Combination of PCPA and Pargyline on the Sexual Performance of Male Rats

| Treatment        | No. of animals tested | No. of animals copulating | No. of animals ejaculating | No. of ejaculations, average $\pm$ s.e. * |
|------------------|-----------------------|---------------------------|----------------------------|-------------------------------------------|
| None             | 30                    | 19                        | 17                         | 5 $\pm$ 0.70                              |
| Pargyline        | 30                    | 3                         | 0                          | 0                                         |
| PCPA + pargyline | 30                    | 30                        | 27                         | 5.9 $\pm$ 1.31                            |
| PCPA             | 30                    | 30                        | 29                         | 4.8 $\pm$ 0.63                            |

\* Number of ejaculations before exhaustion; these values were obtained from animals which ejaculated at least once.

Control and experimental animals were tested at the same time. For each experiment forty rats were divided into four equal groups. The first group was not treated. The second group received 80 mg/kg of pargyline intraperitoneally 8 h before the mating test. The third group received 100 mg/kg of PCPA methylester intraperitoneally daily for 4 days, the last injection 12 h before the mating test. The fourth group received the combination of PCPA and pargyline according to the dose schedules of the second and third group. Table 1 compares the sexual performance of the four groups of animals. The values reported were derived from three experiments. Sixty-three per cent of the control animals copulated at least once and 53% ejaculated. Animals which ejaculated did so with a mean of 5  $\pm$  0.7 ejaculations. On the other hand, only three out of thirty animals treated with pargyline copulated and none ejaculated. Pretreatment with PCPA not only prevented but also reversed the inhibitory effect of pargyline. All animals treated with the combination of PCPA and MAOI copulated and 90% ejaculated. The mean number of ejaculations did not differ statistically from the control group. Treatment with PCPA alone also markedly increased the percentage of animals copulating and ejaculating in respect to control animals.

At the end of the mating test four randomly chosen animals from each group were killed and their brains were analysed for serotonin and catecholamines (Table 2). As expected<sup>13</sup>, pargyline produced an increase in brain serotonin levels greater than in brain catecholamine levels. PCPA depleted brain serotonin while noradrenaline and dopamine concentrations were not significantly influenced. After administration of pargyline to PCPA treated animals, catecholamines but not serotonin accumulated in the brain. In these animals, however, catecholamine levels rose significantly less than in those receiving pargyline alone, suggesting some alteration in the synthesis or storage process of catecholamines.

**Table 2** Effects of PCPA Alone or in Combination with Pargyline on Content of Brain Amines in Male Rats

| Treatment        | Serotonin   | Noradrenaline | Dopamine    |
|------------------|-------------|---------------|-------------|
| Control          | 0.50 ± 0.01 | 0.48 ± 0.02   | 0.80 ± 0.01 |
| Pargyline        | 1.20 ± 0.02 | 0.68 ± 0.02   | 1.16 ± 0.02 |
| PCPA             | 0.03 ± 0.01 | 0.40 ± 0.02   | 0.75 ± 0.02 |
| PCPA + pargyline | 0.08 ± 0.01 | 0.58 ± 0.01   | 0.83 ± 0.02 |

PCPA was given at the dose of 100 mg/kg intraperitoneally daily for 4 days; last injection 14 h before death. Pargyline (80 mg/kg intraperitoneally) 10 h before death. Each value is the average ± s.e. of twelve determinations on whole brain (minus cerebellum). Serotonin and catecholamine were assayed fluorometrically<sup>15,16</sup>.

Six animals treated with PCPA and six animals treated with the combination of PCPA and pargyline were isolated at the end of the mating test, treated next day with PCPA or with the drug combination and retested for sexual activity with receptive females. None of these animals copulated.

Our data suggest that the inhibition of the sexual behaviour in male rats produced by pargyline is secondary to the accumulation of brain serotonin. In fact, the inhibitory effect of pargyline is not only prevented but reversed by PCPA. Accordingly, in PCPA treated animals pargyline produced an accumulation of catecholamines but not of serotonin. These results support our hypothesis that both brain serotonin and catecholamines control sexual behaviour in male animals, serotonin inhibiting and catecholamines stimulating this behaviour<sup>7,8</sup>.

We did not select males with a high level of sexual activity because the purpose of the experiment was not only to show an antagonism against the inhibitory effect of pargyline, but also to find out whether PCPA and the combination of PCPA and pargyline had a true aphrodisiac effect for male animals as in previous studies where sexual behaviour was studied in male to male interactions<sup>7-11</sup>. The fact that a much larger percentage of animals treated with PCPA and a combination of PCPA and pargyline than controls copulated and ejaculated demonstrates that these drugs are true aphrodisiacs for male rats.

We thus disagree with Whalen and Luttge<sup>14</sup> that PCPA and PCPA plus pargyline are not aphrodisiacs because they do not prolong and intensify male-female sexual interactions.

We are unaware of any definitions of an aphrodisiac more specific than those given by Webster's dictionary "that (as a drug or certain foods) which excites to venery" or by Dorland's *Illustrated Medical Dictionary*: "exciting the sexual impulse; any drug that arouses the sexual instinct". Thus, Whalen and Luttge's definition of aphrodisiac is arbitrary. The only conclusion that can be drawn from their study is that PCPA does not enhance maximal performance. In fact, the seven rats used by these authors in their study were "known" to be vigorous copulators; without PCPA they exhibited an average of 7 ± 0.9 ejaculations during a single test. Whalen and Luttge do not present an upper limit for ejaculation, so it is not certain whether this performance could have been exceeded even with the help of drugs.

In agreement with Whalen and Luttge's study, our data show that PCPA and PCPA plus pargyline have no effect on the number of ejaculations or on sexual exhaustion.

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- 1 Moser, M., Brodoff, B., Miller, A., and Goldman, A. G., *J. Amer. Med. Assoc.*, **187**, 192 (1964).
- 2 Goldberg, L. I., *J. Amer. Med. Assoc.*, **190**, 456 (1964).
- 3 Meyerson, B. J., *Acta Physiol. Scand.*, **63**, Suppl. 241, 1 (1964).
- 4 Meyerson, B. J., *Arch. Intern. Pharmacodyn.*, **150**, 4 (1964).
- 5 Meyerson, B. J., *Ann. Med. Exp. Fenn.*, **46**, 394 (1968).
- 6 Meyerson, B. J., and Lewander, T., *Life Sci.*, **9**, 661 (1970).
- 7 Tagliamonte, A., Tagliamonte, P., Gessa, G. L., and Brodie, B. B., *Science*, **166**, 1433 (1969).
- 8 Gessa, G. L., Tagliamonte, A., Tagliamonte, P., and Brodie, B. B., *Nature*, **227**, 616 (1970).
- 9 Shillito, E. E., *Brit. J. Pharmacol.*, **38**, 305 (1970).
- 10 Tagliamonte, P., Perez-Cruet, J., Tagliamonte, A., and Gessa, G. L., *The Pharmacologist*, **12**, 205 (1970).
- 11 Ferguson, J., Henriksen, S., Cohen, H., Mitchell, G., Barchas, J., and Dement, W., *Science*, **168**, 499 (1970).
- 12 Koe, B. K., and Weissman, A., *J. Pharmacol. Exp. Ther.*, **154**, 499 (1966).
- 13 Neff, N. H., and Tozer, T. N., *Adv. Pharmacol.*, **6A**, 97 (1968).
- 14 Whalen, R. E., and Luttge, W. G., *Science*, **169**, 1000 (1970).
- 15 Bogdanski, D. F., Weissbach, H., and Udenfriend, S., *J. Neurochem.*, **1**, 272 (1957).
- 16 Laverty, R., and Taylor, K. M., *Anal. Biochem.*, **22**, 269 (1968).

## Effect of some Antihistaminic Drugs on Post-coital Antifertility Activity of Oestrogens in Rat

THE antifertility action of oestrogens administered after coitus is well established for several species<sup>1</sup> including man<sup>2</sup>. The type of oestrogen, the dose and the time of administration largely determine the mechanism of the effects; these may include<sup>1</sup> alteration of the rate of tubal passage, interference with endometrial development and implantation, specific blastocidal and abortifacient actions.

Although Shelesnyak has postulated<sup>3</sup> that histamine release is an important mediator in the preparation of the endometrium for nidation, the anticipated antifertility effect of systemically administered antihistaminics is equivocal. We report here an increase in the antifertility effect of oestrogens administered immediately after coitus and given in combination with certain antihistaminic drugs.

Sprague-Dawley rats (Charles River Breeding Laboratories), weighing approximately 210–250 g, were housed and mated at a ratio of one male of proven fertility to six females. The presence of spermatozoa in the vaginal smear was taken as indicative of day 1 of pregnancy. Twenty-four hours later (day 2) a single dose of the oestrogen and/or antihistaminic drug was administered orally. Oestrogens were given in corn oil and antihistaminics in either water or 1% polysorbate 80. Nine to 14 days later the females were killed and the uteri were examined for embryos or implantation sites. Animals with one or more such sites were considered to be pregnant. The number of pregnant animals and the number of implantation sites were counted. Control groups treated with oestrogen or with oil alone were included in each set of experiments and several of these were repeated. Because there were no significant differences between these replicates, these results were subsequently pooled. All animals were maintained on Purina laboratory chow and water *ad libitum*.



**Table 1** Effect of Antihistaminics on Post-coital Antifertility Action of Oestrogens in the Rat

| Oestrogen           | Antihistaminic drug | Dose (mg/kg)* | No. of animals | ED <sub>50</sub> † (µg/rat) |
|---------------------|---------------------|---------------|----------------|-----------------------------|
| Diethylstilbesterol | —                   | —             | 80             | 7.8 (7.0–8.7)               |
| Diethylstilbesterol | + Dimenhydrinate    | 75            | 70             | 1.7 (0.9–2.5)               |
| Diethylstilbesterol | + Tripeleennamine   | 30            | 30             | 5.8 (5.7–5.9)               |
| Diethylstilbesterol | + Meclizine         | 25            | 30             | 7.2 (6.2–8.4)               |
| Diethylstilbesterol | + Promethazine      | 30            | 30             | 8.2 (7.6–8.9)               |
| Diethylstilbesterol | + Chlorpheniramine  | 10            | 27             | 8.4 (7.1–9.9)               |
| Ethinyl oestradiol  | —                   | —             | 104            | 53 (50–56)                  |
| Ethinyl oestradiol  | + Dimenhydrinate    | 75            | 72             | 24 (19–30)                  |
| Mestranol           | —                   | —             | 93             | 0.27 (0.24–0.31)‡           |
| Mestranol           | + Dimenhydrinate    | 75            | 65             | 0.19 (0.18–0.21)‡           |

Doses of oestrogens were 5, 10, and 15 µg/rat for diethylstilbesterol, 30, 60, and 90 µg/rat for ethinyl oestradiol and 0.2, 0.3, and 0.45 mg/kg for mestranol.

\* Administered as single oral dose with oestrogen on day 2. A minimum of ten animals given three to five doses were used to establish each ED<sub>50</sub>.

† Calculated as percentage reduction of the fertility index, that is:  $\frac{\text{Number of animals pregnant on section} - \text{mean number of implants in animals pregnant on section}}{\text{Number of animals assumed pregnant}} \times 100$

‡ mg/kg.

Table 1 summarizes the results obtained with three oestrogens and five antihistaminic drugs. Groups of rats which received a fixed dose of the antihistaminic drug were given graded doses of oestrogens. An ED<sub>50</sub> was calculated with 95% confidence limits from the percentage reduction of the fertility index relative to the vehicle controls. The antifertility action was effective with the three oestrogenic compounds alone administered as a single dose on day 2 of pregnancy. Diethylstilbesterol (DES) was the most potent, followed by ethinyl oestradiol and mestranol in order of decreasing effect. The antifertility ED<sub>50</sub> of DES was significantly ( $P < 0.05$ ) reduced by the concurrent administration of dimenhydrinate or tripeleennamine, the latter being approximately three times less effective in the dose tested. Promethazine, meclizine and chlorpheniramine were ineffective in the doses used. Dimenhydrinate also significantly reduced the ED<sub>50</sub> of ethinyl oestradiol and mestranol and with the non-steroidal oestrogens it was even more effective. None of the antihistaminics, when given in the same dose, had any significant effect on the fertility index, nor did 8-chlorotheophylline which forms approximately 45% of the molecular weight of dimenhydrinate. During the experiment we noticed that implantation sites in the horns of the uterus were not evenly distributed, as in untreated animals, but tended to congregate toward the ossa uteri opening into the vagina. In the animals which were killed at 14 days most of the embryos were viable and appeared to be normal.

It is usually accepted that oestrogens administered on the first or second day after coitus accelerate the tubal passage of the ovum so that it reaches the uterus before the endometrium is prepared for nidation<sup>1</sup>. Tubal blocking which may result from relatively large doses (that is, oestradiol 250 µg) has not been observed in the rat<sup>4</sup>. Normally the ovum remains in the tube for approximately 3–4 days and then in the uterus until implantation on the fifth to seventh day. Administration of oestrogen may cause the ovum to reach the uterus as much as 30 h early, when the uterus is not prepared<sup>5</sup>. Dickman and Noyes observed<sup>6</sup> that an ovum 1 day younger than the cornua would degenerate. Greenwald demonstrated<sup>7</sup> that all ova were expelled through the tubes and uterus in 48 h

after a single oral dose of oestradiol cyclopentylpropionate given 24 h after mating.

It seems therefore that antihistaminic drugs enhance the oestrogen-induced increase in the tubal passage of the ovum. Although the mechanism of this action by antihistaminics is not clear, it is not caused by any additive toxic effect of the drugs on the embryo. Dimenhydrinate, the most potent agent, had no deleterious effect on pregnancy in the rat at the same dose, even when administered throughout gestation<sup>8</sup>. Sulman noted<sup>9</sup> that tripeleennamine among other antihistaminics had no effect on pregnancy in the mouse, and chlorpheniramine, considered by Naranjo and Naranjo<sup>10</sup> to be the most embryotoxic drug of the three they tested, had no effect in our experiment. Harper also reported<sup>11</sup> no effect with various antihistaminics on pregnancy in the rat.

Finally, as has been pointed out<sup>12–14</sup>, it is not certain that the antihistaminic property of these drugs is responsible for the observed enhancement of oestrogen activity, for several other potent antihistaminics were ineffective. Further experiments are in progress to assess the mechanism of the observed effect.

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- Saunders, F. J., *Physiol. Rev.*, **48**, 601 (1968).
- Morris, J. McL., and Van Wagenen, G., *Amer. J. Obst. Gynecol.*, **96**, 804 (1966).
- Shelesnyak, M. C., *Rec. Prog. Hormone Res.*, **13**, 269 (1957).
- Greenwald, G. S., *Anat. Rec.*, **157**, 163 (1967).
- Burdick, H. O., and Whitney, R., *Endocrinology*, **21**, 637 (1937).
- Dickman, Z., and Noyes, R. W., *J. Reprod. Fertil.*, **1**, 197 (1960).
- Greenwald, G. S., *Endocrinology*, **69**, 1068 (1961).
- McColl, J. D., Globus, M., and Robinson, S., *Toxicol. Appl. Pharmacol.*, **7**, 409 (1963).
- Sulman, F. G., *Bull. Res. Council Israel*, **4**, 400 (1955).
- Naranjo, P., and Naranjo, E., *Arzneimittel-Forsch.*, **18**, 188, (1968).
- Harper, M. J. K., *J. Reprod. Fertil.*, **9**, 359 (1965).
- Bovet-Nitti, F., Bignami, G., and Bovet, D., *Life Sci.*, **5**, 303 (1963).
- King, G. T. G., *Science*, **141**, 353 (1963).
- Kameswaran, L., Pennefather, J. N., and West, G. B., *J. Physiol.*, **164**, 138 (1962).

## Dietary Migraine and Tyramine Metabolism

MIGRAINE afflicts some 10–15% of the population, which includes a subgroup of dietary migraine sufferers, all of whom specifically exclude certain foods from their diet. When Hanington<sup>1</sup> studied this group extensively all subjects reacted adversely to chemically pure tyramine. Indeed, an oral dose of 100 mg of the pure chemical precipitated a migraine attack entirely indistinguishable from the classic migraine of the subjects concerned<sup>1,2</sup>. Because of this significant correlation, we decided to investigate the metabolism of tyramine in normal and dietary migraine subjects using large doses of tyramine and, separately, tracer doses of <sup>14</sup>C-tyramine.

Twenty-four hour urine samples were collected while subjects were on an unrestricted but drug-free diet which was normal to the individual except that foods known to contain tyramine were not permitted. This was necessary because many of the migraine patients automatically avoided one or other of these foods and it was thought best for the

normals to avoid such foods also. The first early morning urine was voided and the collection was begun. On the second day the first early specimen was taken as the last of the collection; a capsule containing 100 mg of tyramine (as 125 mg tyramine HCl) was taken and the next collection was begun. For the migraine patients, the first urine collection was only acceptable if the day was entirely free of migraine, but for the second collection most (nine out of twelve) reported a migraine of varying severity, as expected; none of the eight normals experienced any reaction to tyramine. A 24 h specimen was chosen even though we realized that most of the compound would be metabolized in 8–12 h in most subjects because it seemed likely that the substantially different rates of elimination could then be ignored; the correctness of this view was born out by the work with radioactive tracers.

For the tracer studies 100 mg of sodium sulphate anhydrous was placed in an empty gelatine capsule together with 5 mg of tyramine and then 0.2 ml. of aqueous solution containing 10  $\mu$ Ci of  $^{14}$ C-tyramine (labelled in the 2 position, specific activity 20  $\mu$ Ci/ $\mu$ mol) was slowly pipetted on to the sulphate so that it was adsorbed and the powder remained dry. The closed capsule was stored in the cold until required but for periods not in excess of 4–6 weeks. The subjects were volunteers over the age of 45, the control group consisting chiefly of friends of the patients. Capsules were ingested in the early morning and specimens of urine were collected 1, 2, 4, 8, 12, 24 and 48 h later; recovery of total radioactivity was substantially complete in the first 24 h. This is in complete agreement with our work on rats in which more than 95% was recovered from the urine in 24 h; no activity was found as  $^{14}$ CO<sub>2</sub> even after 5 days of collection and less than 5% appeared in the faeces (unpublished observations, I. S., P. M. and S. E. March).

Tyramine was determined using Oates's extraction procedure<sup>3</sup> followed by further chromatographic separation<sup>2</sup> to allow a "true" tyramine figure rather than a total "nitroso-naphthol-reactors" figure to be measured. *p*-Hydroxyphenylacetic acid (HPAA) was measured by the method of Karoum *et al.*<sup>4</sup>. Originally free tyramine and free HPAA were measured, but the recovery was only 62% (47–68%) and 69% (54–93%) of the 100 mg of tyramine administered for the normal controls and the dietary migraine patients respectively. Because recoveries were so low we sought metabolites, in particular after acid hydrolysis, and found that the increase in the urinary tyramine plus HPAA was sufficient to account for almost all the previously unrecovered portion of the dose—36% (18–45%) in the controls and 23% (1–42%) in the patients. Of course, other metabolites such as octopamine may have been present but they could have accounted for only a very small fraction of the total. The results are shown in Table 1.

On a normal diet, migraine patients excreted significantly less tyramine, free and conjugated, than did normal individuals

in the same conditions. Likewise, after the 100 mg dose of tyramine, migraine patients still excreted less tyramine than did normals, although, of course, total excretion rose in both groups. For HPAA, however, there was no significant difference between the two groups for either free or conjugated HPAA. All the tyramine differences were significant at greater than the 1% level ( $P < 0.01$ ) whereas none of the HPAA differences were significant at the 5% level ( $P > 0.05$ ).

The data show that, after the ingestion of 100 mg of tyramine, excretion of free tyramine increases by 0.1 mg, that is 0.1% of the dose. If the total urinary tyramine were of dietary origin, therefore, it would correspond to an intake of some 500 mg per day. This cannot be so, for otherwise the patient would suffer continuously from a migraine of the most severe kind. We deduce from this that urinary tyramine is endogenously derived. It follows also that ingested tyramine must be rapidly conjugated or oxidatively deaminated, for there is no evidence for high levels of circulating tyramine. The data also show that conjugation is an important metabolic pathway for both phenolic amines and acids and we suggest that, for quantitative studies, the levels of conjugates should always be measured whenever such phenolic amines are investigated; obvious cases would be in dopa-dopamine studies in Parkinson's disease and phenolic ethanols and glycols.

The apparent identity of levels of free and conjugated HPAA in the urine would suggest that the concentrations of monoamine oxidase and aldehyde oxidase in the liver, kidney and gut are normal in migraine, which is at variance with the postulated<sup>1</sup> low concentration of gut monoamine oxidase in this condition. Conversely, the significantly different concentrations of tyramine conjugates, both before and after the oral load, suggested that the enzymes worthy of further investigation were the conjugases. To ensure that true metabolism, undiluted with dietary variations, was being investigated the experiments with isotopically labelled tyramine were begun. Whole and acid-hydrolysed urines were first examined by direct chromatography on paper using a two-way radiochromatogram scanner capable of locating as little as 500 c.p.m. in a single spot. The most obvious difference after one-way chromatography was that the urine of controls gave a reasonably strong spot in the position of authentic tyramine-O-sulphate whereas the urine of dietary migraines gave either a much smaller or a vanishing small spot in this position; in all cases this spot disappeared after acid hydrolysis. Limited studies with a sulphatase- $\beta$ -glucuronidase preparation greatly reduced the activity of this spot while simultaneously increasing the activity of the spot in the tyramine position.

On the basis of this evidence we postulate that in dietary (tyramine) migraine, the patient suffers from a deficiency in the enzyme responsible for the sulphate conjugation of tyramine. Although there have been comparatively few studies on the metabolism of tyramine in man, other amines have been examined in both man and animals<sup>5</sup>. Little has been made of the importance of the role of conjugation although many amines are known to be metabolized to some extent in this way. Thus Häggendal<sup>6</sup> investigated the conjugation of adrenaline; Sandler and his colleagues have shown that isoprenaline is excreted chiefly as the sulphate<sup>7</sup>; the met-adrenalines<sup>8</sup> and 4-hydroxy-3-methoxyphenylglycol<sup>9</sup> are also excreted predominantly as their sulphates, and Goodall and Alton<sup>10</sup> have found sulphated urinary metabolites after the injection of dopamine into healthy humans.

The fact that much less radioactive tyramine-O-sulphate is found in the dietary migraine urine after a tracer dose and yet, after a 100 mg dose of tyramine, some 10% is excreted in a conjugated form suggests that there is another conjugation pathway for this compound. Both these pathways are quantitatively minor in the normal individual although, after taking an amine oxidase inhibitor, he must probably be capable of increasing the quantity passing these ways. Nevertheless if one of these pathways is defective it might be dangerous to treat the dietary migraine sufferer with such inhibitors.

**Table 1** Urinary Free and Conjugated Tyramine and *p*-Hydroxyphenylacetic Acid

|                | Before tyramine load |                 | Additional      |                 |         |          |
|----------------|----------------------|-----------------|-----------------|-----------------|---------|----------|
|                | Normal               | Migraine        | Normal          | Migraine        | Normal  | Migraine |
|                | mg/24 h              | mg/24 h         | mg/24 h         | mg/24 h         | mg/24 h | mg/24 h  |
| Tyramine free  | 0.8 $\pm$ 0.14       | 0.5 $\pm$ 0.04  | 0.4 $\pm$ 0.17  | 0.1 $\pm$ 0.04  |         |          |
| Tyramine conj. | 2.2 $\pm$ 0.67       | 0.5 $\pm$ 0.08  | 15.3 $\pm$ 1.09 | 10.5 $\pm$ 1.19 |         |          |
| HPAA free      | 20.9 $\pm$ 3.72      | 13.6 $\pm$ 2.23 | 70.1 $\pm$ 5.17 | 77.3 $\pm$ 5.62 |         |          |
| HPAA conj.     | 6.7 $\pm$ 1.55       | 4.3 $\pm$ 0.66  | 23.1 $\pm$ 5.19 | 13.9 $\pm$ 3.91 |         |          |

Urinary concentrations of free and conjugated tyramine and HPAA on a normal diet and after a capsule containing 100 mg T had been swallowed. Total excretion after capsule is the sum of the (before and additional) columns.



The discovery of the pathway of a metabolic defect does not itself illuminate the symptoms or toxic effects. It is possible that extra p-hydroxyphenylacetaldehyde produced in the amine oxidase pathway is highly toxic in its own right and hence a cause of headache. Indeed, one might compare the antabuse therapy for alcoholism which results in an increase of acetaldehyde to toxic proportions and a manifestation of signs and symptoms not unlike those of migraine. Again the reaction of endogenously produced aldehyde with an amine to produce an alkaloid-like substance would be consistent with the views of Davis and Walsh<sup>11</sup> in relation to dopamine forming tetrahydropapaveroline due to derangement or change in aldehyde oxidase pathways.

This evidence suggests that we may be dealing with an inborn error of metabolism. Should this be proved by further studies then this dietary migraine, with an incidence in the region of 1% in the general population, will be the most common error known, being present by one to two orders of magnitude more than any other inborn error recorded.

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<sup>1</sup> Hanington, E., in *Background to Migraine, Second Migraine Symp.* (edit. by Smith, R.) (Heinemann, London, 1967).

<sup>2</sup> Smith, I., Kellow, A. H., and Hanington, E., in *Background to Migraine, Third Migraine Symp.* (edit. by Cochrane, A. L.), 134 (Heinemann, London, 1970).

<sup>3</sup> Oates, J. A., in *Methods of Medical Research* (edit. by Quastel, J. H.), **9**, 169 (Year Book Medical Publications, Chicago, 1961).

<sup>4</sup> Karoum, F., Ruthven, C. R. J., and Sandler, M., *Clin. Chim. Acta*, **20**, 427 (1968).

<sup>5</sup> Lemberger, L., Klutch, A., and Kuntzmann, R., *J. Pharmacol. Exp. Ther.*, **153**, 183 (1966).

<sup>6</sup> Häggendal, J., *Acta Physiol. Scand.*, **59**, 255 (1963).

<sup>7</sup> Morgan, C. D., Ruthven, C. R. J., and Sandler, M., *Clin. Chim. Acta*, **26**, 381 (1969).

<sup>8</sup> LaBrosse, E. H., and Mann, J. D., *Nature*, **185**, 40 (1960).

<sup>9</sup> Axelrod, J., Kopin, I. J., and Mann, J. D., *Biochim. Biophys. Acta*, **36**, 576 (1959).

<sup>10</sup> Goodall, McC., and Alton, H., *Biochem. Pharmacol.*, **17**, 905 (1968).

<sup>11</sup> Davis, V. E., and Walsh, M. J., *Science*, **167**, 1005 (1970).

## Transmission of Double Stranded RNA Viruses to a Strain of *Penicillium stoloniferum* through Heterokaryosis

ALTHOUGH double stranded RNA virus-like particles stimulating interferon production in animals have been discovered in several species of moulds<sup>1-3</sup>, infectivity which is a criterion normally required for a virus has not yet been demonstrated. A reason for this may have been the absence of morphological characteristics in moulds related to the presence of virus. In the work reported here, heterokaryosis was chosen as the method most likely to transmit the particles and to permit the recovery of newly infected colonies.

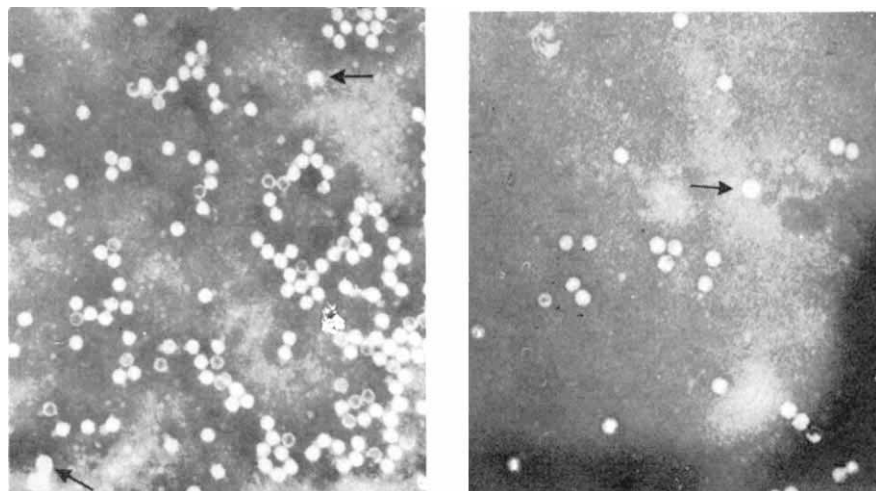
The virus-infected strain used was *Penicillium stoloniferum* strain ATCC 14586 in which three types of particles have been found<sup>4</sup>. The recipient strain was a white-conidiated mutant obtained from *P. stoloniferum* strain ATCC 10111. Strains were grown and virus preparations were obtained by a method used before<sup>1</sup>.

Heterokaryotic conidiophores, developing on agar medium from a mixture of conidia from both parental strains, produced



**Fig. 1** Polyacrylamide gel electrophoresis in TAE buffer (0.04 M Tris, 0.05 M acetate, 0.0016 M EDTA, pH 8.0). Anode at bottom of gel, 6 mA/tube (internal diameter 5 mm), 2 h, stained with Coomassie blue. Virus bands are indicated by arrows. Left, infected parental strain; centre, newly infected strain (note the proportionally much lighter fast band); right, virus-free parental strain.

**Fig. 2** Electron micrographs of virus preparation from infected parent (left) and from newly infected strain (right), stained with potassium phosphotungstate ( $\times 75,000$ ). Virus particles were 25–30 nm in diameter, except a few (less than 1%), indicated by arrows, which were 32–35 nm in diameter. The total concentration in the newly infected strain was about 10% of the concentration in the infected parent.



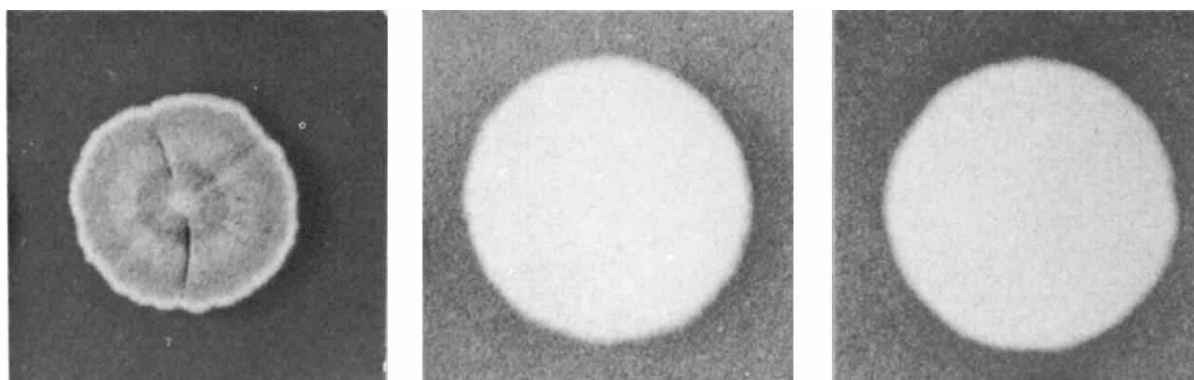


Fig. 3 Colonies of *P. stoloniferum* grown on agar medium: virus-infected strain (left); the originally virus-free strain shows the same growth rate and morphological growth pattern before (centre) and after (right) infection.

conidia of both types. Conidia taken from them were spread for isolation and the white colonies obtained were tested for virus. Out of six heterokaryotic conidiophores, four gave infected white colonies; this proportion shows how easily heterokaryosis can transmit particles to a virus-free strain.

Presence or absence of virus was tested by polyacrylamide gel electrophoresis<sup>4</sup> (Fig. 1). In the virus-free parent, no bands were detected in the region where virus bands (slow and fast moving) are found in the infected parent and in the newly infected strain. The presence of virus was confirmed by electron microscopy (Fig. 2); the low virus concentration, which was found unchanged after two subcultures of the newly infected strain, seems to be a characteristic of the new host.

Inoculations on agar medium were made to test for differences in morphology and growth rate due to the presence of virus (Fig. 3). Although the irregular morphology and the low growth rate of the infected parent have been attributed to the presence of virus<sup>3,5</sup>, it was impossible to correlate morphological features or growth rates with virus infection.

Infectivity has also been demonstrated by the same technique in a visible mutant of another virus-free strain of *P. stoloniferum* (strain CMI 91966) and in *Aspergillus niger*<sup>6</sup>.

These results show that heterokaryosis is an effective method for demonstrating susceptibility of moulds to double stranded RNA viruses. In the case reported here, the newly infected strain had a lower total concentration of virus and relatively less fast virus than the infected parent. However, because the concentrations of the types of virus seem to be dependent on the host-virus relationship, it is probable that other newly infected strains will be found with a higher content of virus than the parental strain or with different proportions of the virus types. Experiments are in progress to establish the mechanism of infection with fungal viruses.

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<sup>1</sup> Banks, G. T., Buck, K. W., Chain, E. B., Darbyshire, J. E., Himmelweit, F., Ratti, G., Sharpe, T. J., and Planterose, D. N., *Nature*, **227**, 505 (1970).

<sup>2</sup> Banks, G. T., Buck, K. W., Chain, E. B., Darbyshire, J. E., and Himmelweit, F., *Nature*, **223**, 155 (1969).

<sup>3</sup> Hollings, M., and Stone, O. M., *Sci. Prog., Oxf.*, **57**, 371 (1969).

<sup>4</sup> Buck, K. W., and Kempson-Jones, G. F., *Nature*, **225**, 945 (1970).

<sup>5</sup> Banks, G. T., Buck, K. W., Chain, E. B., Himmelweit, F., Marks, J. E., Tyler, J. M., Hollings, M., Last, F. T., and Stone, O. M., *Nature*, **218**, 542 (1968).

<sup>6</sup> Lhoas, P., *Aspergillus News Letter*, **11**, 8 (1970).

## Streptovaricins inhibit RNA Dependent DNA Polymerase Present in an Oncogenic RNA Virus

REPORTS by Temin<sup>1</sup> and Baltimore<sup>2</sup> indicate that an RNA dependent DNA polymerase is present in oncogenic RNA viruses. These reports, now confirmed and extended<sup>3-6</sup>, provide a mechanism by which an RNA virus can insert stable genetic information into a host chromosome. Most recently, Gallo *et al.*<sup>7</sup> have reported an RNA dependent DNA polymerase, apparently analogous to that of the RNA tumour viruses, in lymphoblasts of leukaemic patients. This enzyme is strongly inhibited by N-demethylrifampicin, and to a lesser extent by rifampicin<sup>7</sup>. The potential chemotherapeutic importance of these findings clearly warrants an immediate survey of related compounds which may inhibit, perhaps selectively, these "reverse transcriptases". The relationship, if any, between cell growth characteristics and the continued presence of this novel enzyme is not yet known, but the implications are clear.

The streptovaricins, like the structurally similar rifamycins<sup>8</sup>, inhibit DNA dependent RNA polymerase from bacteria<sup>9,10</sup> but not from Ehrlich ascites cells<sup>10</sup> or liver nuclei<sup>11</sup>. RNA polymerase seems to represent the major, perhaps only, target in the bacterial cell for these two families of antibiotics, and various studies, including examinations of drug-resistant mutants<sup>12</sup>, argue strongly for similar mechanisms of action. Thus, on the basis of the actions of the streptovaricins in bacteria analogous to the rifampicins, we tested the streptovaricins for possible inhibitory activity against tumour virus RNA dependent DNA polymerases. These antibiotics, unlike the rifampicin derivatives, are already available in relatively large quantities and, indeed, preliminary toxicological studies in primates have already been made (unpublished data of the Upjohn Co.). We now report that the streptovaricin complex is an extremely potent inhibitor of the reaction by which DNA is transcribed from the RNA template resident in purified murine leukaemia virions. Streptovaricin C and rifamycin SV are also quite active, while streptolydigin and rifampicin are relatively poor inhibitors.

The Moloney strain of murine leukaemia virus was grown in JLS-V9 cells and purified by I. Toplin (Electronucleonics, Inc., Bethesda) using a double sucrose gradient centrifugation (the K and B29 rotors)<sup>12</sup>. Electron microscopy of the purified fractions revealed  $2-3 \times 10^{11}$  particles/ml.; the protein concentration was 0.5-0.75 mg/ml. The virus stocks could be stored at 4° C in 8% glycerol for at least 1 week without loss of polymerase activity.

The general characteristics of the MLV polymerase reaction were similar to those which were reported before<sup>1-6</sup>. Linear incorporation rates were seen generally for approximately 25 min in the case of the resident viral RNA (with rA·rU, linear rates were obtained for at least 60-90 min); optimal activity



required all four deoxynucleoside triphosphates, and virion pretreatment with pancreatic RNAase A markedly decreased the rate of the reaction primed by the endogenous template.

**Table 1** Effect of Streptovaricins on MLV RNA Dependent DNA Polymerase

| Exp. | Treatment                         | Inhibitor conc. (μg/ml.) | Initial reaction rate ( $V_0$ ) (pmoles TTP incorp./4 μg viral protein/15 min) | "Net synthesis" (pmoles/4 μg viral protein/30 min) | % inhibition (calculated at 15 min) |
|------|-----------------------------------|--------------------------|--------------------------------------------------------------------------------|----------------------------------------------------|-------------------------------------|
| 1    | None                              | None                     | 1.95                                                                           | 3.0                                                | 0                                   |
| 2    | [2% DMSO]                         | None                     | 1.95                                                                           | 3.15                                               | 0                                   |
| 3    | Streptovaricin (complex) (U-7750) | 20                       | 1.17                                                                           | 2.20                                               | 40%                                 |
|      |                                   | 40                       | 0.45                                                                           | 0.75                                               | 77%                                 |
|      |                                   | 100                      | 0.45                                                                           | 0.75                                               | 77%                                 |
|      |                                   | 400                      | 0.63                                                                           | 0.75                                               | 68%                                 |
| 4    | Streptovaricin A (U-9344)         | 400                      | 1.25                                                                           | 2.25                                               | 33%                                 |
| 5    | Streptovaricin C (U-8741)         | 20                       | 2.15                                                                           | 3.30                                               | 0                                   |
|      |                                   | 400                      | 0.54                                                                           | 0.87                                               | 73%                                 |
| 6    | Streptolydigin (U-5481)           | 400                      | 1.87                                                                           | 3.12                                               | 4%                                  |
| 7    | Rifampicin (Dow)                  | 400                      | 1.50                                                                           | 2.75                                               | 13%                                 |
| 8    | Rifamycin SV (Le Petit)           | 20                       | 1.94                                                                           | 3.15                                               | 0                                   |
|      |                                   | 400                      | 0.45                                                                           | 0.75                                               | 77%                                 |

The reaction mixtures (105 μl.) contained 60 mM KCl, 40 mM Tris-HCl (pH 7.8), 2 mM MnCl<sub>2</sub>, 2 mM dithiothreitol, 0.02% Triton-100, 0.1 mM dATP, dGTP, dCTP, 0.65 nmol <sup>3</sup>H-methyl-TTP (9,000 c.p.m./μmol, New England Nuclear Corp.) and 4 μg of viral protein. The mixture was incubated at 37° C and acid-precipitable radioactivity was determined in 20 μl. aliquots (withdrawn after 2, 15, 30 and 60 min). Since only a small fraction of the input radioactivity was incorporated into DNA, special precautions were taken to diminish non-incorporated ("trapped") counts. The method of Weiss *et al.*<sup>14</sup>, developed in the measurement of polynucleotide ligase, was used. Briefly outlined, the aliquot was added to 12 μg of "carrier" DNA (1 ml. in saturated phosphate) and an equal volume of 16% trichloroacetic acid (in saturated phosphate) was then added. After 25 min at 4° C, the precipitate was collected on glass fibre paper (Whatman GF/C, 2.4 cm, presoaked in saturated phosphate) retained on a porcelain Hirsch funnel and exhaustively washed. This procedure regularly reduces the "background" radioactivity to 45–50 d.p.m. Radioactivity was determined in a liquid scintillation counter with an efficiency of 27%. All "inhibitors" were prepared in 100% DMSO and kept in the dark at 4° C. Control experiments established that the highest concentrations of DMSO (final concentration 2%, see experiment 2) or glycerol (final concentration 1.6%) used in the reaction mixtures, either singly or together, did not influence the rate of the reaction. Streptovaricin complex is a mixture of macrolides: A : C : (B + F + G) : (D + E) with a ratio of 16.3 : 50 : 16.3 : 16.3. Each of these reactions had reached a plateau phase by 30 min; the term "net synthesis" is applied to the amount of product formed by this time.

The results of inhibition studies are shown in Table 1. The streptovaricin complex, a mixture of seven macrolides (plus undetermined components), is a potent inhibitor of the reaction. More than 75% inhibition is obtained with concentrations as low as 40 μg/ml. Direct comparisons of this activity with the N-demethylrifampicin inhibition of leukaemic polymerase<sup>7</sup> suggest, at the very least, a similar order of sensitivity. (Such comparisons might be misleading since parameters such as *km* and [enzyme] and [template] optima are not yet known in these systems.) Complete inhibition is not obtained, however, even with concentrations of 400 μg/ml. Streptovaricin C, a pure macrolide, was also quite active but less so (on a weight basis) than the complex. Because streptovaricin C represents about 50% of the streptovaricin complex (see legend to Table 1), we can assume that other molecules, as yet not precisely identified, account for the major proportion of the extremely strong inhibitory activity of the complex. Studies are under way to determine whether the major streptovaricins present (A to G) or trace products are responsible for the inhibition.

Rifamycin SV gave an inhibition similar in magnitude to that of streptovaricin C. Since this rifampicin analogue lacks any antipox activity—presumably because it does not have the

hydrazone side chain apparently essential for antipox activity of rifampicin<sup>15</sup>—it would seem that this molecule must have an intrinsically different action on the MLV "transcriptase". As can be seen, rifampicin and streptolydigin, both potent inhibitors of the bacterial DNA dependent RNA polymerases, were relatively inactive.

In other experiments (not shown) the reaction (in the absence of inhibitors) was linear for about 35–40 min; interestingly, in the presence of streptovaricin C and rifamycin SV, incorporation of deoxynucleotide was slower and reached a plateau after 15–20 min. The streptovaricin complex inhibited the initial rate of the reaction without any detectable early onset of the plateau phase. These results could also suggest that certain inhibitors may block initiation of transcription. Experiments to test this hypothesis, in which the time of addition of the inhibitors is varied, are under way. Since these compounds may block initiation of transcription, the sequence in which the enzyme, template and inhibitor are added to the reaction mixture could explain whether or not complete inhibition is achieved.

To have any substantial chemotherapeutic promise, the compounds we have mentioned must have a selective inhibitory function. To this end, we (W. A. C., W. W. B. and C. Steuart) are evaluating the effects of these antibiotics on the different polymerases present in normal and leukaemic lymphocytes. It should be possible to determine rather quickly if a selective inhibition can be achieved with this family of compounds. It also seems most important to determine if the reversion frequencies of cells, already transformed by RNA tumour viruses, change under the pressure of polymerase blockade.

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*Note added in proof.* Direct comparisons indicate that the streptovaricin complex is more active than N-demethylrifampicin against the RNA dependent DNA polymerase of MLV.

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- 1 Temin, H. M., and Mizutani, S., *Nature*, **226**, 1211 (1970).
- 2 Baltimore, D., *Nature*, **226**, 1209 (1970).
- 3 Spigelman, S., Burny, A., Das, M. R., Keydar, J., Schlom, J., Travnick, M., and Watson, K., *Nature*, **227**, 1029 (1970).
- 4 Green, M., Rokutanda, M., Fujinaga, K., Ray, R. K., Rokutanda, H., and Gurgu, C., *Proc. US Nat. Acad. Sci.*, **67**, 385 (1970).
- 5 Hatanaka, M., Huebner, R. J., and Gilden, R. V., *Proc. US Nat. Acad. Sci.*, **67**, 143 (1970).
- 6 Todaro, G., Zeve, V., and Aaronson, S. A., *Nature*, **226**, 1047 (1970).
- 7 Gallo, R. C., Yang, S. S., and Ting, R. C., *Nature*, **228**, 927 (1970).
- 8 Rinehart, K. L., Coverdale, C. E., and Martin, P. K., *J. Amer. Chem. Soc.*, **88**, 3150 (1966).
- 9 Hartman, G., Honikel, K. O., Knüsel, F., and Nüesch, J., *Biochim. Biophys. Acta*, **145**, 843 (1967).
- 10 Mizuno, S., Yamazaki, H., Nitta, K., Uehara, R., and Umezawa, H., *Biochim. Biophys. Acta*, **157**, 322 (1968).
- 11 Shmerling, Z. G., *Biochim. Biophys. Res. Commun.*, **37**, 965 (1969).
- 12 Yura, T., and Igarashi, K., in *Progress in Antimicrobial and Anticancer Chemotherapy*, **2**, 405 (University Park Press, Baltimore, Maryland, 1970).
- 13 Toplin, I., *Applied Microbiol.*, **15**, 582 (1967).
- 14 Weiss, B., Live, T. R., and Richardson, C. C., *J. Biol. Chem.*, **243**, 4530 (1968).
- 15 Zakay-Rones, Z., and Becker, Y., *Nature*, **226**, 1162 (1970).

## Heavy Metals and the Fertilization of Rainbow Trout Eggs

THE effects of poisons on the development and survival of fertilized fish eggs have often been described but little information is available on the effects of such substances on either the gametes or fertilization. Mann, for example, has reported<sup>1</sup> that a dodecylbenzenesulphonate detergent caused a 26% loss of mobility of trout spermatozoa at a concentration of 5 mg l.<sup>-1</sup>, and the anaesthetic drug tricaine methanesulphonate was found<sup>2</sup> not to affect fertilization provided that the concentration did not exceed 50 mg l.<sup>-1</sup>. The effects of heavy metals on these life stages and processes do not, however, seem to be known, even though these poisons are common pollutants of river waters in industrial areas. We report here results of preliminary tests made at this laboratory into the effects of copper and nickel on the fertilization of eggs of the rainbow trout, *Salmo gairdneri* Richardson.

The eggs were obtained by section of the ovaries of a freshly killed female fish weighing 555 g. Spermatozoa were obtained from a 400 l. volume of water (of total hardness 260 mg l.<sup>-1</sup> as CaCO<sub>3</sub>, pH 7.8, and temperature 8° C) into which a single male fish had been induced to discharge milt.

Three samples (2 l.) of this water were taken in borosilicate glass beakers and a solution of copper sulphate or of nickel sulphate was added immediately to two of the samples to give metal ion concentrations of 1.0 mg Cu<sup>++</sup> l.<sup>-1</sup> in one and 1.0 mg Ni<sup>++</sup> l.<sup>-1</sup> in the other; the third samples served as a control. The eggs, after removal from the ovary, were distributed in sub-equal lots between the three treatments. These operations were completed within a period of 5 min. The beakers were left undisturbed for the next 30 min, after which time each batch of eggs was removed and placed in a separate aquarium (from which light was excluded) in a fast, continuous flow of clean, well aerated, hard water (total hardness 260–280 mg l.<sup>-1</sup> as CaCO<sub>3</sub>, copper 0.5 µg l.<sup>-1</sup>, nickel nil) at a temperature of about 9° C. The number of eggs used and the percentages fertilized and hatching in each treatment are given in Table 1. The values of  $\chi^2$  obtained from comparison of the numbers fertilized in the control with those fertilized in the two metals are also given.

**Table 1** Fertilization of Rainbow Trout Eggs in Solutions of Copper and Nickel Salts

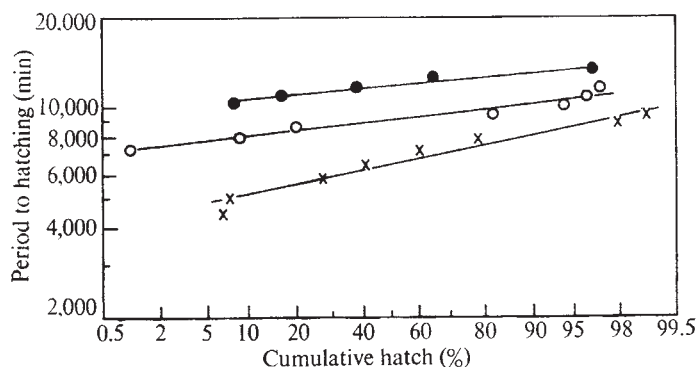
| Treatment                              | No. of eggs | % fertilized | $\chi^2$ | Fertilized eggs hatching (%) |
|----------------------------------------|-------------|--------------|----------|------------------------------|
| 1 mg Cu <sup>++</sup> l. <sup>-1</sup> | 389         | 22.4         | 1.78     | 97                           |
| 1 mg Ni <sup>++</sup> l. <sup>-1</sup> | 419         | 23.4         | 2.88     | 99                           |
| Nil (control)                          | 332         | 18.0         | —        | 100                          |

The hatching rates of eggs from the three treatments are shown in Fig. 1. Observations were made daily at 0900 h and 2000 h up to the start of hatching. The time origin in the figure is the time of the last observation, 35 days after fertilization, at which all the eggs were still intact. The periods to median hatch (on the same time scale) are given in Table 2. The data relating time to percentage hatch were analysed by the methods of Litchfield<sup>3</sup>.

The proportion of eggs fertilized was low but it compares reasonably with those obtained elsewhere by methods involving ovarian section<sup>4</sup>. The  $\chi^2_{(1)}$  values indicated that the differences observed between each treatment and the control were not statistically significant ( $P > 0.05$ ). Fertilization in the metal

**Table 2** Period of Time from 0900 h on Day 35 to Median Hatch

| Treatment     | Period to median hatch (min) | 95% confidence limits (min) | Slope function |
|---------------|------------------------------|-----------------------------|----------------|
| Nil (control) | 11,500                       | 11,320–11,680               | 1.07           |
| Nickel        | 9,600                        | 8,850–9,150                 | 1.09           |
| Copper        | 6,400                        | 6,170–6,640                 | 1.19           |



**Fig. 1** Hatching of rainbow trout eggs following fertilization in solutions of copper and nickel. The period of hatching was measured from the time, 0900 h 35 days after fertilization, when all eggs were last observed to be intact. ●, Control; ○, nickel; ×, copper.

solutions did, however, increase the rate of development, particularly in the case of copper, and the eggs from this treatment all hatched before any had done so in the controls. The rate of hatching of the eggs fertilized in the copper solution was also significantly different ( $P < 0.05$ ); it was slower than that of the control eggs or of those fertilized in the nickel solution—the slopes of the curves of these last two were similar.

Because the concentrations of copper and nickel used here exceed those typically found in British rivers, even those badly polluted, it seems that, at least in hard waters, neither of these two poisons is likely to be responsible for any impairment of fertilization among trout. This does not, however, imply that successful development would occur under conditions of continuous exposure to these poisons. It is not known whether the differences observed in the development have a biological significance.

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<sup>1</sup> Mann, H., *Münchn. Beitr. Abwass.-, Fisch.- Flussbiol.*, **9**, 131 (1967).

<sup>2</sup> *Fish Cult. Bull.*, **2**, (1), 5 (1969).

<sup>3</sup> Litchfield, J. T., *J. Pharmac. Exp. Ther.*, **97**, 399 (1949).

<sup>4</sup> Brown, V. M., and Templeton, W. L., *Nature*, **203**, 1257 (1964).

## Cyanide Insensitive Culture Form of *Trypanosoma brucei*

ON the basis of ultrastructural and cytochemical observations, Vickerman has suggested<sup>1–3</sup> that the single mitochondrion of sleeping sickness trypanosomes undergoes a cyclical activation and repression during the life cycle of the parasite. Detailed studies of the respiratory metabolism of this group of trypanosomes have been confined to bloodstream stages and forms from established *in vitro* cultures<sup>4–7</sup>. The culture forms are believed to correspond to the midgut stage of the tsetse fly vector<sup>8</sup>. The bloodstream forms lack cytochromes and a functional Krebs cycle<sup>7</sup>; terminal respiration is cyanide insensitive and is mediated largely by extramitochondrial L-α-glycerophosphate oxidase<sup>6</sup>. Established culture forms have cyanide sensitive terminal respiration and a full functional Krebs cycle<sup>7</sup>.

We describe here a culture form of *Trypanosoma brucei* which develops during the transformation of bloodstream forms into established culture forms and differs from either of these forms in its respiratory metabolism.



Rats infected with a pleomorphic strain of *T. brucei* were bled 1 day after the first peak of parasitaemia. Trypanosomes were separated from the blood by centrifugation, were washed once with sterile Hanks solution and were then transferred to Thompson bottles containing 500 ml. of the human blood lysate broth of Pittam<sup>9</sup>. The cultures were incubated at 26° C. The trypanosomes began growing immediately, and their doubling time was approximately 17 h. Morphological transformation from bloodstream to "culture" form took place in this medium, and the exponential phase of growth continued for 80 h.

Cultures of newly transformed organisms were collected, washed, suspended in 0.32 M sucrose, 24 mM Tris, 1 mM EGTA (ethylene glycol-bis-( $\beta$ -aminoethyl ether) N,N'-tetraacetic acid) at pH 7.4 and were then broken in a hand operated all-glass homogenizer. The oxidation of succinate, NADH and L- $\alpha$ -glycerophosphate (L- $\alpha$ -GP) by whole homogenates was followed polarographically with an oxygen electrode.

**Table 1** Development of Succinic Oxidase Activity in Transforming Trypanosomes

| Time in culture | O <sub>2</sub> uptake with succinate<br>(10 mM) (nmol O <sub>2</sub> /h/mg<br>protein) | % inhibition by<br>KCN (up to 10 mM) |
|-----------------|----------------------------------------------------------------------------------------|--------------------------------------|
| 0 (bloodstream) | < 10                                                                                   | 0                                    |
| 24 h            | 90                                                                                     | 0                                    |
| 48 h            | 220                                                                                    | 0                                    |
| 4 days          | 220                                                                                    | 0                                    |
| 14 days         | 116                                                                                    | 21                                   |

In addition to cyanide, the oxidation of succinate, NADH and L- $\alpha$ -GP by homogenates of cultures up to 4 days old was completely insensitive to azide (5 mM) and to antimycin A (8 nmol/mg protein): the oxidation of NADH and L- $\alpha$ -GP by such homogenates was also completely insensitive to amytal (3 mM) and to rotenone (5 nmol/mg protein).

The cyanide insensitive succinoxidase activity of the culture forms was, however, sensitive to inhibition by malonate and also by diphenylamine, which is a known inhibitor of cyanide insensitive respiration in plant systems<sup>10,11</sup>; 1 mM gave a minimum of 60% inhibition of the cyanide insensitive succinoxidase. 100% of the activity of the cyanide insensitive succinoxidase of homogenates was sedimentable by centrifugation at 15,000g for 15 min.

These studies suggest that in these culture forms which are cyanide insensitive it is very unlikely that electrons derived from succinate pass to oxygen by way of a conventional electron transport chain which is cytochrome mediated. The cyanide insensitive oxidation of NADH and L- $\alpha$ -GP by newly transformed culture forms could be accounted for by the operation of the L- $\alpha$ -GP oxidase system described by Grant and Sargent<sup>6</sup>. The oxidase system responsible for the cyanide insensitive oxidation of succinate, unless linked in some way to the L- $\alpha$ -GP oxidase, is completely unknown, and work is now in progress on the purification and characterization of this novel oxidase system.

We have found that newly transformed trypanosomes were unable to survive repeated subculture beyond 16 days into the blood lysate broth of Pittam<sup>9</sup>. If the medium was diluted with fresh broth lacking the blood lysate, or the organisms were washed and transferred to the freshly prepared biphasic medium of Tobie, von Brand and Mehlman<sup>12</sup>, the trypanosomes continued to grow and were capable of prolonged subculture in a form with cyanide sensitive respiration. Transfer of washed bloodstream trypanosomes directly into biphasic medium resulted in the death of large numbers of the transferred organisms, followed by slow initial growth rate of the surviving organisms compared with those transferred directly into the blood lysate broth.

It seems likely that for a culture of *T. brucei* to be established in a form capable of surviving repeated subculture, it must first pass through a culture form which is initially insensitive

to cyanide before it develops into the sensitive form which will grow in media containing a lower concentration of blood. This cyanide sensitive form reverts back to a form showing cyanide insensitive respiration if transferred to a medium containing the higher concentration of blood lysate<sup>13</sup>.

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- <sup>1</sup> Vickerman, K., *Trans. Roy. Soc. Trop. Med. Hyg.*, **56**, 487 (1962).
- <sup>2</sup> Vickerman, K., *Nature*, **208**, 762 (1965).
- <sup>3</sup> Vickerman, K., in *Ecology and Physiology of Parasites* (edit. by Fallis, A. M.) (University of Toronto Press, in the press).
- <sup>4</sup> Flynn, I. W., and Bowman, I. B. R., *Trans. Roy. Soc. Trop. Med. Hyg.*, **62**, 132 (1968).
- <sup>5</sup> Fulton, J. D., and Spooner, D. F., *Exp. Parasit.*, **8**, 137 (1959).
- <sup>6</sup> Grant, P. T., and Sargent, J. R., *Biochem. J.*, **76**, 229 (1960).
- <sup>7</sup> Ryley, J. F., *Biochem. J.*, **85**, 211 (1962).
- <sup>8</sup> Thomson, J. E., and Sinton, J. A., *Ann. Trop. Med. Parasitol.*, **6**, 331 (1912).
- <sup>9</sup> Pittam, M. D., *Comp. Biochem. Physiol.*, **33**, 127 (1970).
- <sup>10</sup> Baker, J. E., *Arch. Biochem. Biophys.*, **103**, 148 (1963).
- <sup>11</sup> Sharpless, T. K., and Butow, R. A., *J. Biol. Chem.*, **245**, 58 (1970).
- <sup>12</sup> Tobie, E. J., Brand, T. von, and Mehlman, B., *J. Parasitol.*, **36**, 48 (1950).
- <sup>13</sup> Brown, R. C., and Evans, D. A., *Trans. Roy. Soc. Trop. Med. Hyg.* (in the press).

## Resistance to Water Movement in Tomato Plants infected with *Fusarium*

PATHOGENS causing vascular wilt diseases grow in the xylem tissues of their host and induce severe wilting of the foliage, possibly by causing an impediment in the movement of water in the xylem<sup>1,2</sup>. Measurements of water flux through stem segments have indicated that the xylem resistance of infected plants may be increased twenty-fold<sup>3,4</sup>. Xylem resistance is normally so low, however, that such an increase would not be expected to result in wilting<sup>5</sup>. Furthermore, there has been no clear demonstration that a decrease in water potential is associated with the observed wilting. The measurements of transpiration and leaf water potential reported here show that *Fusarium oxysporum* Schlecht. f.sp. *lycopersici* (Sacc.) Snyder and Hans. causes an increase in the xylem resistance of tomato (*Lycopersicon esculentum* Mill.) sufficient to account for leaf wilting.

Tomato plants, variety 'Bonny Best', were grown in soil and inoculated 4-5 weeks after seeding by the root dip technique<sup>6</sup>. Plants were used for experiments when the first symptoms of water stress appeared, 10-16 days after inoculation. A preliminary experiment indicated that leaves on infected plants wilted at the same leaf water potential as leaves on healthy plants from which water was withheld.

The terminal leaflet of the fourth adult leaf was enclosed in a chamber equipped with a beta-gauge and differential psychrometer so that leaf water content and transpiration measurements could be made<sup>7</sup>. The plant was reduced to a leaflet plus petiole and stem by excising the stem under degassed water at ground level, and by cutting off other leaves and leaflets. The stem and petiole outside the chamber were covered with plastic. Light intensity and vapour concentration in the chamber were adjusted to yield a steady transpiration rate and count rate on the beta-gauge. It was assumed that steady state conditions had been achieved when during 30 min there was less than a 2% change in transpiration and relative water content<sup>8</sup>. The petiole was then excised

under water next to the stem and a new steady state transpiration rate was measured after the system was adjusted to give the original count rate. The procedure was repeated after the petiole was excised under water 2 cm from the experimental leaflet. At the end of the experiment, the leaflet was removed from the chamber at the original count rate, washed and blotted dry in a humid chamber, and placed in a thermocouple psychrometer for the measurement of water potential.

In this experiment, the transpirational flux could be expressed by the equation

$$F = \frac{-\Delta\Psi}{R} = \frac{\Delta c}{r}$$

where  $\Delta\Psi$  is the water potential gradient in the fluid phase,  $R$  is the resistance in the fluid phase,  $\Delta c$  is the vapour concentration gradient between the leaf and bulk air, and  $r$  is the resistance in the vapour phase<sup>9</sup>. Thus  $R$  can be calculated from  $F$  ( $\text{g s}^{-1}$ ) and  $\Delta\Psi$  (bars) without reference to  $r$ , which includes stomatal resistance.  $R$  was calculated for each of the steady states I have described, and resistances of the stem and petiole, which are in series with leaf resistance, were obtained by difference.

The resistance of healthy stems and petioles was consistently low (Table 1), and the observed resistances were not very different from those predicted by Poiseuille's law<sup>5</sup>, for example, 8–12 and 112–330  $\text{bar s g}^{-1}$  predicted for the stems and petioles respectively. The leaflet offered the chief resistance in the above ground portion of healthy plants. If one assumes a transpiration rate of  $5 \times 10^{-6} \text{ g cm}^{-2} \text{ s}^{-1}$  ( $1.8 \text{ g dm}^{-2} \text{ h}^{-1}$ ), the average leaflet resistance of  $55.7 \times 10^3 \text{ bar s g}^{-1}$  would cause a water potential drop of almost 7 bars. Thus the leaflet resistance reported here is somewhat higher than is generally assumed for leaves, but less than the resistance of  $1.5 \times 10^5 \text{ bar s g}^{-1}$  calculated from Boyer's<sup>10</sup> observations on expanding sunflower leaves. There is other evidence that leaf resistance is high<sup>11</sup>, such as the large difference in water potential between adjacent transpiring and nontranspiring leaves found by Rawlins<sup>9</sup>.

**Table 1** Resistance to Water Movement in Healthy and Infected Tomato Plants

| Plant    | Appearance of the leaf* | Resistance ( $\text{bar s g}^{-1} \times 10^3$ ) |         |          |
|----------|-------------------------|--------------------------------------------------|---------|----------|
|          |                         | Stem                                             | Petiole | Leaflet† |
| Healthy  | Turgid                  | 0                                                | 0       | 47.8     |
|          |                         | 0.3                                              | 0.3     | 72.2     |
|          |                         | 0.2                                              | 0.3     | 62.6     |
|          |                         | 0.1                                              | 0       | 56.3     |
|          |                         | 0                                                | 0.2     | 39.8     |
| Infected | Turgid<br>Wilted‡       | 52.7                                             | 0.1     | 52.4     |
|          |                         | 111.6                                            | 81.8    | 116.2    |
|          |                         | —                                                | ∞       | 126.3    |
|          |                         | —                                                | ∞       | 206.3    |
|          |                         | —                                                | ∞       | 416.7    |

\* Observed at the start of the experiment.

† Includes the resistance of the remaining 2 cm of petiole.

‡ Infected plants are in order of decreasing turgor.

In the infected plant where the experimental leaf appeared turgid, stem resistance was more than 500 times that observed in healthy stems, but petiole and leaflet resistances were normal. In infected plants, however, where the experimental leaf appeared wilted, petiole resistance was very high. The resistance was taken to be infinite where leaflet water content would remain constant only when there was no transpiration. These leaflets did not take up water until the petiole was cut near the chamber; after cutting, their water content increased markedly. Dimond and Waggoner<sup>12</sup> also found that leaflets which wilted while disease was developing recovered only when excised from the petiole. Leaflet resistance was high where petiole resistance was high, probably because the leaflet measurements included xylem resistance within the leaflet and in the remaining 2 cm of petiole. Pieces of wilted leaf tissue from healthy and disused plants gained in water content with equal speed when floated on water.

It is reasonable to conclude from our data that *Fusarium* causes a large increase in xylem resistance and that this increase can induce wilting when it occurs in the petiole, and perhaps in the leaflet, where xylem resistance is normally higher than elsewhere in the plant<sup>5</sup>.

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- Dimond, A. E., *Ann. Rev. Plant. Physiol.*, **6**, 329 (1955).
- Talboys, P. W., in *Water Deficits and Plant Growth* (edit. by Kozlowski, T. T.), 255 (Academic Press, New York, 1968).
- Beckman, C. H., Kuntz, J. E., Riker, A. J., and Berbee, J. G., *Phytopathology*, **43**, 448 (1953).
- Ludwig, R. A., *MacDonald Coll. Tech. Bull.*, No. 20 (McGill University, Montreal, 1952).
- Dimond, A. E., *Plant Physiol.*, **41**, 119 (1966).
- Scheffer, R. P., and Walker, J. C., *Phytopathology*, **43**, 116 (1953).
- Jarvis, P. G., and Slatyer, R. O., *Planta*, **90**, 303 (1970).
- Jarvis, P. G., and Slatyer, R. O., *Science*, **153**, 78 (1966).
- Rawlins, S. L., in *Stomata and Water Relation in Plants* (edit. by Zelitch, I.), 69 (Conn. Agric. Exp. Sta. Bull. 664, 1963).
- Boyer, J. S., *Plant Physiol.*, **43**, 1056 (1968).
- Boyer, J. S., *Science*, **163**, 1219 (1969).
- Dimond, A. E., and Waggoner, P. E., *Phytopathology*, **43**, 619 (1953).

## Investigation of Drag Reduction by Certain Algae

INVESTIGATIONS were carried out between March and September 1969 to determine the drag reduction effects produced by certain species of algae in No. 3 towing tank at the Ship Division of the National Physical Laboratory. The tank is 48 feet wide, 25 feet deep and 1,300 feet long and contains the water added in August–September 1957. Small additions are regularly made to compensate for evaporation losses. The tank is constantly used for hydrodynamical experiments and is daily skimmed by towing a plastic tube along the tank to remove surface scum. The tube extends across the width of the tank and below it is suspended a muslin curtain. Samples from this scum were studied and species were tested for drag reduction effects.

Initially, the algae were not isolated from the samples. Five litres of water from the "dock area" of the No. 3 towing tank were treated with about 3 g of  $\text{MgCl}_2$ , illuminated continuously, and bubbled with  $\text{CO}_2$ . After about 8 days the culture was brilliant green, and microscopic examination showed that this was almost entirely due to one unicellular alga, *Chlorella vulgaris*. Other species of algae were isolated and cultured in a relatively pure form<sup>1,2</sup>. Filamentous blue-green algae (for example *Oscillatoria* sp.) were isolated from the samples using a fine platinum wire and then transferred to "E+S" agar with continuous sub-culturing to reduce contamination to a minimum. The virtually pure cultures were transferred to Beijerinck's liquid media. Unicellular algae (for example *Chlorella vulgaris*) were isolated using a micro-pipette of bore 0.01 mm. The cells were transferred to "E+S" agar and sub-cultured; when sufficiently pure they were transferred to a suitable liquid medium. Cultures of certain algal species were obtained from the Botany School, Downing Street, Cambridge. These algal species were selected because they had previously been found<sup>3,4</sup> to give significant



drag reduction. The isolated cultures were also used for the identification of species isolated. Normal sterilizing procedures were adapted to reduce contamination of cultures to a minimum. The relative concentrations of the algae in the cultures were determined using standard techniques.

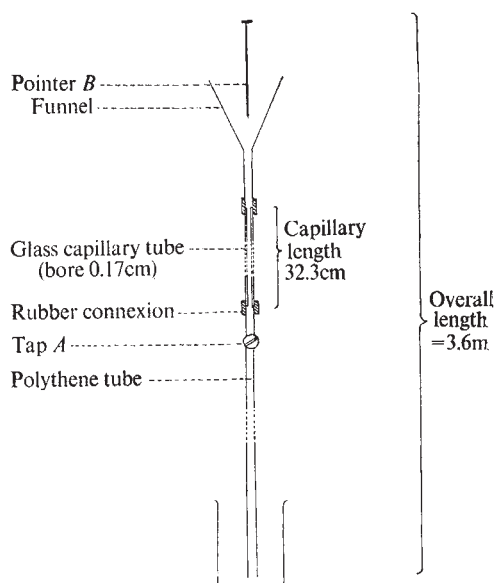


Fig. 1 Apparatus used to investigate drag reduction.

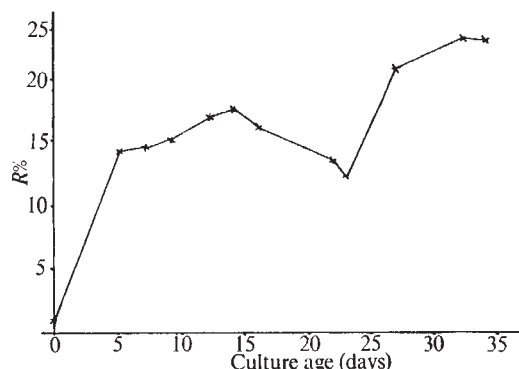


Fig. 2 Graph plotted from results obtained from No. 3 tank culture, May-June.

The apparatus used to measure drag reduction is shown in Fig. 1. Initially, the apparatus was filled with water with tap A open and the beaker in position to maintain a constant pressure head. The level of the water in the funnel was adjusted until the tip of pointer B just touched the water surface. The temperature of the culture to be tested was noted and the water to be used in the next part of the experiment was adjusted to this. Water (200 ml.) was poured into the funnel and its time of turbulent flow through the glass capillary tube was measured using a stop watch ( $tw$ ). The experiment was repeated and an average value ( $tw'$ ) obtained. The time of flow of 200 cc. filtered algal culture was measured in a similar way and an average value ( $ta'$ ) obtained. From these results the drag reduction ( $R\%$ ) for the algal culture was expressed by:

$$R\% = \frac{(tw' - ta')}{tw'} \times 100$$

The apparatus was thoroughly rinsed before another culture was tested. All algal cultures were filtered because algal

cellular material masked some drag reduction effects produced by the algal secretions.

Negligible drag reduction was obtained with certain algal species, which Hoyt<sup>3,4</sup> had found to cause drag reduction. Samples of these cultures were tested at the Admiralty Research Laboratories, Teddington, using a more refined apparatus developed by Dr Ritter. Turbulent flow in this apparatus is faster than in our gravity driven one and the flow conditions thus approximate more closely to those produced in Hoyt's turbulent rheometer; our results were nevertheless confirmed.

The results of these studies are summarized in Figs. 2-4. Drag reduction was maximal at 17.7% when the liquid culture was 13 days old and brilliant green (Fig. 2). A second peak drag reduction of 23.3% occurred on the 34th day. Samples of water taken from the No. 3 tank at other times and cultured in this way gave similar results (Fig. 3).

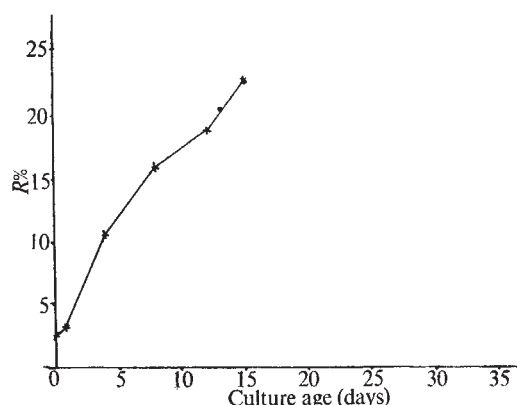


Fig. 3 Graph plotted from results from No. 3 tank culture, August-September.

It is possible that these fluctuations are caused by *Chlorella vulgaris* which at some phases of its life cycle secretes copious amounts of mucus which would liberate into the water polysaccharides which could cause drag reduction. Bacterial metabolites reduce drag in some cases<sup>5</sup>. Destruction of the algal cell wall provides conditions favourable to bacterial growth and the second peak may be due to bacterial metabolites. None of the algal cultures was in fact totally axenic. Cultures of *Chlorella vulgaris* (211/1i) and *Porphyridium cruentum* (13800/1a) also increased drag reduction (Fig. 4). *P. cruentum* was not found in any of our samples, but Hoyt<sup>3</sup> claims that this species reduces drag, and our results confirmed this.

Among the species for which we could not confirm Hoyt's finding of drag reduction was *Porphyridium aerugineum*

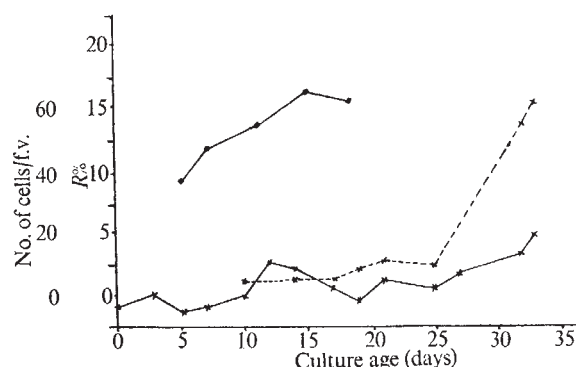


Fig. 4 Graph plotted from results obtained from algal cultures. ●—●, *Porphyridium cruentum*; ×—×, *Chlorella vulgaris*; ×---×, number of cells/field of vision (×45 objective).

(1380/2). Preliminary tests with *Porphyridium cruentum* had also failed to show drag reduction. None of the cultures was axenic, and so it is possible that any polymer secreted by the algae was destroyed by bacteria. This is, however, unlikely to account for the results of preliminary tests using *P. cruentum*, which has antibacterial properties. Probably in this case growth was retarded by a nutrient deficiency which was rectified in a later successful culture.

Most of the algal species isolated from the No. 3 tank and cultured and tested were found to give negligible drag reduction. It is quite possible that different strains of species would give higher drag reduction effects. The highest value obtained for *C. vulgaris* was 5% and that for *P. cruentum* was 16.8%. There are, however, more than sixty strains of *C. vulgaris* alone.

Drag reduction estimates using isolated algal species were smaller than those obtained from culturing No. 3 tank water. This suggests that drag reduction is the result of the combined effect of several bacterial and algal species or alternatively that the algae mainly responsible have not as yet been isolated.

Maximum concentrations recorded for our cultures of *Anacystis nidulans*, *Chlorococcum multinucleatum*, *Navicula* sp. and *Porphyridium aeruginosum* were three, thirty-four, eight and nine respectively per field of vision of a  $\times 45$  objective, and these concentrations gave negligible drag reductions. *P. cruentum*, on the other hand, gave a drag reduction of the order of 15% at a concentration of one cell per field of vision and is thus a much more effective drag reducing agent than the other species investigated.

The tank conditions are continuously changing and for this reason some species temporarily become more abundant than others. Thus the rotifer *Keratella cochlearis* was abundant in the scum samples during August–October 1959, but had not previously been recorded except in 1967. There is a need for further investigation to determine the conditions when drag reducing algae and other organisms become abundant. Even when drag reducing algae are relatively abundant in the tank, however, their concentration is normally far too small to produce measurable drag reduction in the raw tank water, and could only become sufficient to give the sort of values obtained in the cultured samples in quite exceptional conditions, never so far encountered.

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<sup>1</sup> Pringsheim, E. G., *Pure Cultures of the Algae* (Cambridge University Press, 1946).

<sup>2</sup> Chu, S. P., *Growth of Planktonic Algae*.

<sup>3</sup> Hoyt, J. W., and Silo, G., *Science*, **149**, 1509 (1965).

<sup>4</sup> Hoyt, J. W., *Proc. Eleventh Intern. Towing Tank Conf.*, 88 (1966).

<sup>5</sup> Kenis, P. R., *Nature*, **217**, 940 (1968).

## Addressed Exponential Delay Line Theory of Cochlear Organization

SINGLE unit studies of the auditory nerve, such as that of Tasaki<sup>1</sup>, have shown that the neurones innervating the cochlea are in general sharply tuned, each to its own characteristic

frequency (CF). In response to a sharp click a unit of mid or low CF tends to fire repetitively and at preferred times, as if stimulated by a poorly damped resonator very sharply tuned to its CF<sup>2</sup>. In contrast, the travelling wave generated as the mechanical response to a pure tone, as measured by von Békésy<sup>3</sup>, has a relatively flat amplitude envelope and the impulse response for the basilar membrane computed on the basis of von Békésy's data indicates that the system is highly damped<sup>4</sup>. We propose a fresh approach, not based on the principle of mechanical resonance<sup>5-7</sup>.

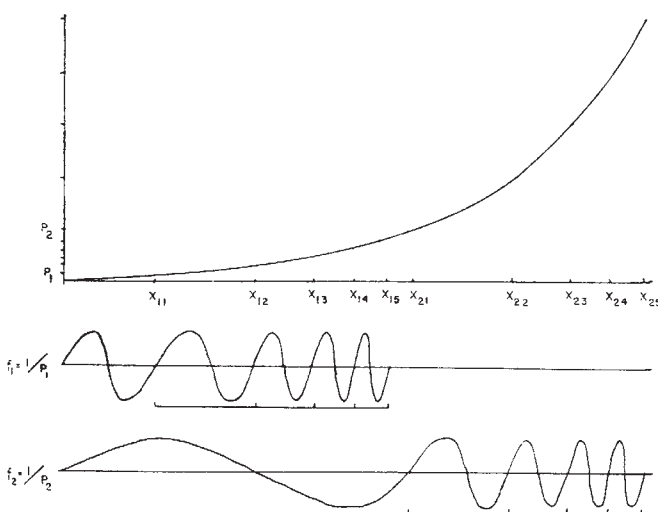


Fig. 1 Over the portion of the delay line where the delay function is truly exponential the vibration patterns for all sinusoidal input frequencies are congruent, that is they differ only by longitudinal displacements. In particular, the  $\{x_i\}$  point sets corresponding to oriented zero crossings of the input signals have the same pattern of intervals for all input frequencies. This result is shown for two frequencies  $f_1$  and  $f_2$ . The first pattern point for each frequency corresponds with a delay of one cycle of that frequency. Because all patterns are the same except for position, only one basic pattern recognition configuration is needed; replication of the one configuration on successive overlapping intervals along the delay line will suffice precisely to identify frequencies covering a wide frequency band.

Available evidence strongly supports the idea that, with regard to phase information, the cochlear mechanical response closely corresponds to that of an exponential delay line over most of its length. Von Békésy's original measurements of phase angle of the mechanical response as a function of frequency at points along the basilar membrane not too near the apex show a slope of very nearly  $2\pi$  per octave for frequencies higher than the best frequency at each point<sup>3</sup>. For lower frequencies Flanagan and Bird's<sup>8</sup> more reliable calculated minimum phase delay curve is close to that expected on the assumption of an exponential delay. Tasaki, Davis and Legoux<sup>9</sup> recorded the cochlear microphonic from several points along the length of the cochlea and determined, at each distal point, the frequencies which showed a phase shift of  $\pi$  and  $2\pi$  radians from the response recorded from the most basal point. The plots of delay versus distance along the cochlea show a very close fit to an exponential function for both measures. Finally, Nordmark *et al.*<sup>10</sup> showed that microphonic waves and hence, presumably, mechanical waves of all frequencies seem to travel with the same velocities.

A simple exponential delay line is defined by equation (1) which relates the propagation time, or delay,  $\tau$ , to any point  $x$  on the line, for  $x \geq x_b$ ,

$$\tau = ae^{kx} \quad (1)$$

The constant  $x_b$  defines a neighbourhood of the origin where



the function departs from the exponential. Solving for  $x$  we have

$$x = \frac{1}{k} \ln \tau - \frac{1}{k} \ln a \quad (2)$$

For any  $P = 1/f$ , provided the frequency  $f$  is sufficiently great, we can then define a point set  $\{x_i\}$ , where

$$x_{nP} = \frac{1}{k} \ln P + \frac{1}{k} \ln n - \frac{1}{k} \ln a \quad (3)$$

The coordinate  $x_{nP}$  defines the point exactly  $2n\pi$  radians out of phase with the origin for the frequency  $f$ . Equation (3) means that the wavelengths of the second, third, and so on, complete cycles of the wave pattern for frequency  $f$  are independent of  $f$ . Or, more precisely, for all frequencies  $f$  with  $P = 1/f \geq ae^{kx_b}$ ,

$$x_{(n+1)P} - x_{nP} = \frac{1}{k} [\ln(n+1) - \ln n], n \geq 1 \quad (4)$$

A similar pattern, with different point spacing, can be generated beginning with any arbitrary phase for the  $x_1$  point. Fig. 1 illustrates the constancy of the terminal wave pattern as a function of frequency.

Insofar as the velocity gradient of the cochlea locally approximates that of an exponential delay line, the travelling wave maps the frequency domain on identical terminal wave patterns on the basilar membrane. Thus, for each frequency,  $f$ , within the range of values determined by the line length and velocity gradient, there is a unique set of points  $\{x_i\} = x_1, x_2, \dots, x_n$ , forming a well defined pattern, each member of which is  $2\pi$  radians out of phase with the succeeding member of the series for the given input frequency and for no other frequency. To identify precisely an input frequency it is only necessary to identify the set of pattern points  $\{x_i\}$  which are in phase with respect to their mechanical displacements. A detection mechanism operating for each set of points  $\{x_i\}$  could, for example, look for coincidence of oriented zero crossings at the points of the set.

The anatomy of the organ of Corti suggests a specific organization for such a recognition system. Most afferent nerve fibres innervating the outer hair cells extend for considerable distances along the length of the cochlea<sup>11</sup>. These so called outer spiral fibres could serve both to respond to peaks of the spatial derivative of the travelling wave and to bring together the information for each of the sets of pattern points  $\{x_i\}$ . Fig. 2 illustrates this concept, as well as another critical anatomical feature: the relative number of afferent fibres innervating inner and outer hair cells.

Spoendlin has demonstrated by electron microscopy that most afferent nerve fibres entering the organ of Corti from the foramina in the spiral lamina—the habenula perforata—go directly by a radial course to innervate the nearest inner hair cell<sup>12</sup>. Relatively few afferent fibres would be needed to address any given set of pattern points  $\{x_i\}$  (Fig. 2). Each such set of outer hair cell afferents addressed to a given set of pattern points  $\{x_i\}$  would serve the function of identifying the narrow frequency band generating wave patterns closely matching the defining point set. Whatever mechanism is used to implement this requirement it is clear that little intensity information could be handled in addition by the outer hair cell fibre system. The relatively large number of fibres addressed to the inner hair cells seems appropriate to the requirements of an intensity code based on a distribution of fibre thresholds, requiring only controlled gating to specify peaks in the input frequency spectrum.

The question of frequency specificity of this type of system can best be approached using a highly abstract model. At each point  $x_i$ , a perfect zero crossing detector which excites the

outer hair cell fibre addressed to the point  $x_i$  is assumed. All fibres to a given pattern set are assumed to have essentially the same overall delay times for the conduction of excitation pulses to the loci of interaction; and for the sake of simplicity, an exponential delay line is assumed which extinguishes each pure tone travelling wave pattern to zero beyond the point corresponding to a phase shift of  $5(2\pi)$  radians. Specificity of the temporal excitation pattern for the frequency defined by the  $\{x_i\}$  point set is illustrated in Fig. 3. When input frequency is either slightly higher or slightly lower than the tuned frequency of the point set, the pattern of the excitation onset times for the detector fibres spreads out with intervals between timing events equal to the absolute difference in period lengths. Input frequencies lower than the tuned frequency fire all five detector fibres; but higher frequency wave patterns fail to reach all the detector loci. With either higher or lower frequency input signals, although the synchrony of detector fibre firing is lost the repetition rate of the group firing pattern remains equal to the input frequency.

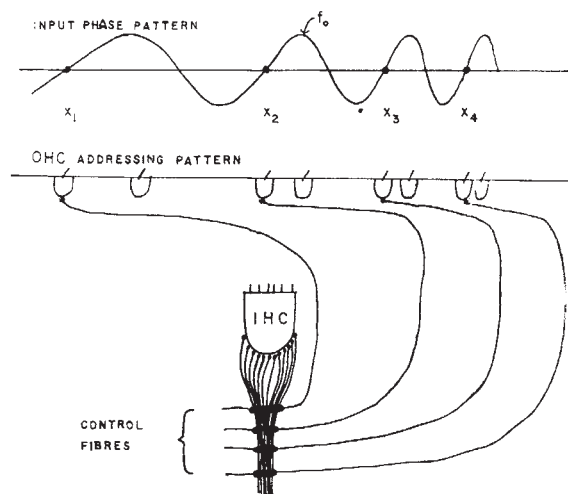


Fig. 2 A suggested neurophysiological realization for a frequency detection assembly tuned to the frequency  $f_0$  is shown diagrammatically along with the instantaneous phase pattern for frequency  $f_0$  on the pattern point set  $\{x_i\}$  for this frequency. The relatively numerous afferent fibres are shown in synaptic contact with a single inner hair cell, IHC. They also receive excitatory synaptic contacts from each of a set of control fibres. The control fibres synapse with outer hair cells, OHCs, only in restricted regions corresponding with the mathematically defined points  $\{x_i\}$  for the frequency  $f_0$ . The control fibres are depolarized at the zero crossing corresponding to the scala tympani–scala vestibuli swing of the basilar membrane. Depolarization effects on an afferent fibre summate at the locus of spike generation somewhat central to the synaptic region under the inner hair cell. It is assumed that the total conduction time to the place of spike generation on afferent fibres is the same for all control fibres.

This elementary form of the sharpening mechanism, based on the phase locked addition of excitatory pulses, would probably only work for relatively low frequencies. What is needed to remedy this situation is something like frequency dependent pulse width which can easily be provided by supplementing the system of excitatory control fibres with a dual system of inhibitory control fibres addressed to loci  $\pi$  radians out of phase with excitatory fibres. With this addition the recognition system may be considered to carry out a running approximate cross-correlation on each outer hair cell fibre subsystem. For this refinement we simply need to implement the requirement for zero crossing detectors with peak spatial derivative detectors. The spatial derivative is also convenient for very high frequency detection—where digitized cross-correlation techniques are both inappropriate and not required, given the

observed sharpness of high frequency travelling wave envelopes<sup>13</sup>.

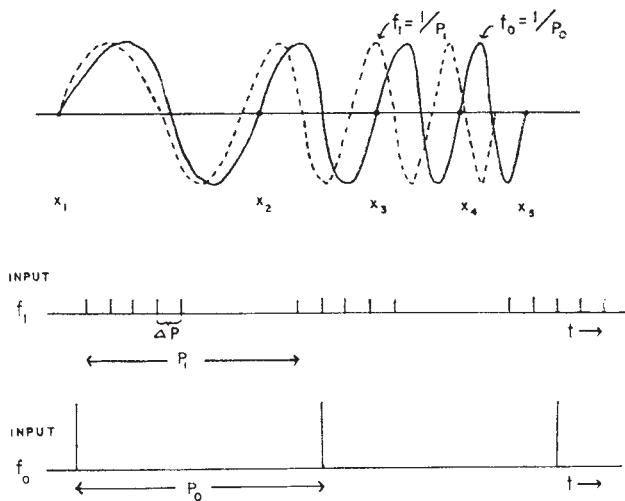


Fig. 3 The basic principle underlying the use of an exponential delay line as a frequency detector is illustrated here. The portion of the delay line containing the pattern point set  $\{x_i\}$  for a given frequency  $f_0$  is shown at the top of the figure along with instantaneous wave patterns for the frequencies  $f_0$  and also  $f_1 > f_0$ . The wave amplitudes are normalized and the instant represented in each case corresponds with the downward going zero crossing at the point  $x_1$  of the  $f_0$  pattern point set. At this instant, by definition, the  $f_0$  wave pattern makes a zero crossing of the same direction at all  $f_0$  pattern points. In contrast, the corresponding zero crossings of the  $f_1$  wave are delayed by time intervals  $\Delta P = |P_1 - P_0|$ ,  $2\Delta P$ ,  $3\Delta P$ , and so on, at  $f_0$  pattern points to the right of  $x_1$ . Thus, if an additive unit pulse is produced on a fibre by each negative going zero crossing at a pattern point the summed detector output pulses for the  $f_0$  pattern point set are as shown; with input  $f_0$  the output pulses summate, which they do not for input  $f_1$ . An equivalent result holds true for frequencies  $f_1 < f_0$ , but the firing order of addressed points is reversed.

The system outlined here will have sharp tuning curves for pure tones, but will have even more sharply tuned impulse responses, as found, for example, by Møller<sup>14</sup>. Short latency inhibitory effects produced by one tone on the response to a second tone may be expected from this model; these also are a feature of unit response data<sup>15-17</sup>. Control fibres may also be assumed to have "spontaneous" activity, so the superposition of these random point sequences will produce a Poisson like output on the inner hair cell afferents—with, of course, a dead time resulting from post firing refractoriness. As Lynn and Sayers<sup>7</sup> have previously pointed out, models with this feature of point sequence superposition give spontaneous outputs with properties correctly matching that recorded from primary auditory nerve fibres. Both physiological<sup>18-20</sup> and behavioural studies<sup>21</sup> show that one of the functions of the extensive efferent system is to protect the hearing mechanism from disruption by background noise. This needed protective feature can easily be incorporated into the proposed system by a feedback inhibition of the control fibre excitation process.

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- <sup>1</sup> Tasaki, I., *J. Neurophysiol.*, **17**, 97 (1954).
- <sup>2</sup> Kiang, N. Y.-S., *Discharge Patterns of Single Fibers in the Cat's Auditory Nerve*, MIT Research Monograph No. 35 (MIT Press, Cambridge, Massachusetts, 1965).
- <sup>3</sup> von Békésy, G., *Experiments in Hearing* (McGraw-Hill, New York, 1960).
- <sup>4</sup> Flanagan, J. L., *J. Acoust. Soc. Amer.*, **34**, 1370 (1962).
- <sup>5</sup> Weiss, T. F., *Kybernetik*, **3**, 153 (1966).
- <sup>6</sup> Huxley, A. F., *Nature*, **221**, 935 (1969).
- <sup>7</sup> Lynn, P. A., and Sayers, B. McA., *J. Acoust. Soc. Amer.*, **47**, Part 2, 525 (1970).
- <sup>8</sup> Flanagan, J. L., and Bird, C. M., *J. Acoust. Soc. Amer.*, **34**, 114 (1962).
- <sup>9</sup> Tasaki, I., Davis, H., and Legoux, J.-P., *J. Acoust. Soc. Amer.*, **24**, 502 (1952).
- <sup>10</sup> Nordmark, J. O., Glatke, T. J., and Schubert, E. D., *J. Acoust. Soc. Amer.*, **46**, Part 2, 1587 (1969).
- <sup>11</sup> Spoendlin, H., *The Organization of the Cochlear Receptor* (S. Karger, Basel, Switzerland, 1966).
- <sup>12</sup> Spoendlin, H., *Acta Otolaryngol.*, **67**, 239 (1969).
- <sup>13</sup> Johnstone, B. M., and Boyle, A. J. F., *Science*, **158**, 389 (1967).
- <sup>14</sup> Møller, A. R., *Acta Physiol. Scand.*, **78**, 299 (1970).
- <sup>15</sup> Katsuki, Y., Suga, N., and Kanno, Y., *J. Acoust. Soc. Amer.*, **34**, 1396 (1962).
- <sup>16</sup> Rupert, A., Moushegian, G., and Galambos, R., *J. Neurophysiol.*, **26**, 449 (1963).
- <sup>17</sup> Frishkopf, L. S., *J. Acoust. Soc. Amer.*, **36**, 1016 (1964).
- <sup>18</sup> Nieder, P., and Nieder, I., *Nature*, **227**, 184 (1970).
- <sup>19</sup> Nieder, P., and Nieder, I., *Brain Res.*, **21**, 135 (1970).
- <sup>20</sup> Nieder, P., and Nieder, I., *Exp. Neurol.*, **28**, 179 (1970).
- <sup>21</sup> Dewson, J. H. III, *J. Neurophysiol.*, **31**, 122 (1968).

## Role of Predators in the Reversal of Imbalances in Microbial Ecosystems

MICROBIAL communities contain a wide variety of microorganisms living together, frequently at high population densities. These communities are relatively stable and not easily disturbed. When a heterogeneous animal or plant population becomes unbalanced by a minor ecological disturbance, such as immigration of a foreign species or by a temporary change in nutrient concentration, the community structure may be altered permanently. By contrast, microbial populations developed either by intrusion of non-indigenous microorganisms or by temporary ecological perturbations are vigorously resisted by the native community. Intermicrobial predators exist in most microbial communities. This communication describes the homeostatic processes by which microbial imbalances are reversed by predators among the native population.

The balance of natural bacterial populations is often upset by the disposal of sewage into natural waters or the soil, which results in the temporary domination of the community by the intestinal bacteria. These bacteria, however, are incapable of competing with the native microbial community. The predominant intestinal bacterium, *Escherichia coli*, survives well in sterile freshwater, seawater or soil. Data in Fig. 1 show that there is a direct proportionality between the numbers of indigenous bacteria in river water and the decline of *E. coli* in that water. This relationship is not unique to freshwaters, and was also detected in seawater<sup>1</sup> and in some soils. The microorganisms involved in the destruction of *E. coli* are common to all habitats investigated. Bacterial predators capable of enzymatically degrading the cell walls developed in large numbers in response to *E. coli* inoculation. But the largest response to the intrusion of foreign bacteria came from two obligately parasitic microorganisms, the parasitic bacterium *Bdellovibrio bacteriovorus*, and an amoeba of the genus *Vexillifera*. Inoculation of *E. coli* into freshwater, seawater or soil resulted in a stimulation of these organisms from a level of  $10^4/\text{ml}$ . to  $10^6/\text{ml}$ . in 5 days. These populations were unstable



and rapidly declined after the destruction of the immigrant bacteria. In the absence of ineffectual immigrants, old or physiologically inactive native bacteria are probably the nutrient source for these predators.

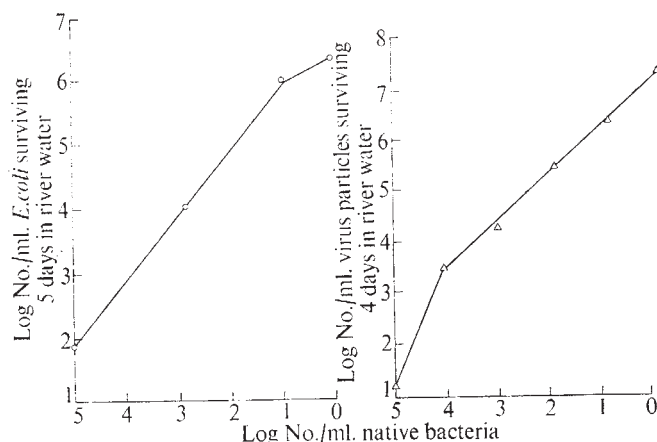


Fig. 1 Relationship between the number of indigenous bacteria in river water and the rate and extent of decline of *E. coli* and bacteriophage  $\Phi$ X-174 in that water.

A similar reversal of an imbalanced community was observed when a terrestrial species of the fungus *Pythium debaryanum* was added to seawater<sup>2</sup>. Mycelial mats of the fungus remained intact in sterile seawater, but disintegrated in natural seawater in 12 days in shake cultures. The predator was an agar-decomposing bacterium of the genus *Agarobacterium* which preyed on the fungus and totally degraded the terrestrial species of *Pythium* in 75 h by lysing components of the cell walls. A marine species of *Pythium* was susceptible to the predator, but was disintegrated at a slower rate. These data suggest that native and foreign microorganisms are unequally susceptible to predation by the indigenous microflora. Non-indigenous fungi with no capacity for growth are more rapidly disintegrated. Physiologically adapted native species are attacked but at least for a time can outgrow the activity of their predators.

indigenous microbial population, and 99% of the *Skeletonema* population decayed in 24 h in the dark in natural seawater. When either *Chlamydomonas* or *Skeletonema* were kept in sterile water in the dark the population declined imperceptibly in 12 days. The predators for both algae were isolated. They were both bacteria of the genus *Pseudomonas*, apparently lysing the algae by enzymatic destruction of the cell walls. Predaceous activity, however, was quite specific. The bacterium active against *Chlamydomonas* preyed on both marine and freshwater species of *Chlamydomonas*, but showed no activity against any other algal genus, or other bacteria, fungi or algae tested. The predator against *Skeletonema* was also genus specific.

Canter and Lund<sup>3</sup> have detected a similar destruction of algal populations in lakes caused by microbial predators. They demonstrated that chytrids parasitic on diatoms and blue-green algae either delayed the time of maximal bloom or decreased the size of the maximum bloom. It would be interesting to know if microbial predators against algae serve to maintain homeostasis by continuously lysing old or competitively weak algae. It is generally assumed that the majority of phytoplankton are consumed by predators at a higher trophic level, but a significant portion of the algal biomass may fall prey to microbial predators before zooplankton or fish have the opportunity to use them.

Viruses have an unusually strong capacity for survival outside the host in spite of their obligate parasitism. The infectious hepatitis virus carried into the sea in sewage survives well and is concentrated in shellfish. The processes controlling survival of the bacteriophage  $\Phi$ X-174 in natural waters have been studied in some detail<sup>4</sup>. In both seawater and river water the rate of inactivation was found to be proportional to the concentration of the native microflora. Typical data for river water are shown in Fig. 1. It is apparent from these data that a portion of the indigenous microbial community is involved in the destruction of the virus. Enrichment techniques, however, have failed to yield any microorganisms capable of preying on the virus. It seems reasonable to assume that antiviral microorganisms are present in natural waters, and presumably in the soil too, but that the minute size of the virus makes isolation of these antagonists extremely difficult.

It is apparent from this study that predator microorganisms comprise an important component of the microflora of natural habitats. These microorganisms act as homeostatic regulators to correct microbial imbalances and restore a balanced environment in both aquatic and terrestrial ecosystems. The microbial predator-prey relationship offers a useful model for the study of animal predation. Microbial communities are diverse and develop rapidly, and the microbial system could be used to study the effects of high population densities on predator-prey relationships or to study the response of predators to ineffectual immigrants. The system also lends itself to the development of more complex models in population dynamics.

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<sup>1</sup> Mitchell, R., Yankofsky, S., and Jannasch, H. W., *Nature*, **215**, 891 (1967).

<sup>2</sup> Mitchell, R., and Wirsén, C., *J. Gen. Microbiol.*, **52**, 335 (1968).

<sup>3</sup> Canter, H. M., and Lund, J. W. G., *New Phytol.*, **37**, 238 (1948).

<sup>4</sup> Mitchell, R., and Jannasch, H. W., *Env. Sci. Technol.*, **3**, 941 (1969).

Table 1 Microbial Destruction of the Freshwater Alga *Chlamydomonas* in Lake Water and of the Marine Alga *Skeletonema* in Seawater

| Time<br>Days | <i>Chlamydomonas</i>             |                                      | <i>Skeletonema</i>             |                                    |
|--------------|----------------------------------|--------------------------------------|--------------------------------|------------------------------------|
|              | Sterile<br>lake water<br>No./ml. | Non-sterile<br>lake water<br>No./ml. | Sterile<br>seawater<br>No./ml. | Non-sterile<br>seawater<br>No./ml. |
| 0            | $4 \times 10^6$                  | $9 \times 10^5$                      | $1 \times 10^5$                | $2 \times 10^5$                    |
| 4            | $4 \times 10^6$                  | $7 \times 10^5$                      | $1 \times 10^5$                | $4 \times 10^3$                    |
| 7            | $2 \times 10^6$                  | $2 \times 10^5$                      | $2 \times 10^5$                | —                                  |
| 11           | $2 \times 10^6$                  | $3 \times 10^3$                      | —                              | —                                  |

In eutrophic waters the algal population is unnaturally enlarged because of the high concentration of nutrients in the water. Ultimately some nutrient becomes limiting and the algal population reaches a maximum level. At this point indigenous microbial predators again respond to a physiologically incapacitated community and restore the ecological balance. Table 1 shows the fate of the freshwater alga *Chlamydomonas* in lake water and of the marine diatom *Skeletonema* in seawater. Both survived well when placed in high concentration in filter-sterilized samples of water from their native habitats and in non-sterile samples in the light. However, 99% of the *Chlamydomonas* population decayed in 11 days in the dark in fresh natural lake water containing an

# BOOK REVIEWS

## Values and Morality

*Human Values and Natural Science.* Edited by Ervin Laszlo and James B. Wilbur. (Proceedings of the Third Conference on Value Inquiry, State University of New York, College at Geneseo.) (Current Topics of Contemporary Thought, Vol. 4.) Pp. xiii + 292. (Gordon and Breach: New York and London, July 1970.) \$15; £6.25.

THE proceedings of conferences and symposia invariably reveal a gap between intention and achievement; rarely can the gap have been wider than in the present case. The editors list the subjects that submitted papers were required to consider as follows:—

- “(1) The relevance of the methods of natural science for the recognition and description of human values.
- (2) The values obtained by placing man as a natural entity into the universe described and presupposed by natural science.
- (3) The possibility and desirability of preserving or inducing values by means of the techniques and applications of natural science in the light of its cognitive results.”

Fortunately perhaps, no reference is made to the third topic in the book; and the first two attract scant attention. No more than a third of the papers make any serious reference to the methods, practices and results of the natural sciences; the primary orientation of the contributors is in fact to problems in philosophy, particularly those relating either to the “naturalistic fallacy” or to the free-will/determinism debate. In both cases the dominant approach is to cast doubt on traditionally accepted distinctions. It is unfortunate that the publishers do not make this philosophical emphasis clear. Instead, a histrionic and irresponsible sleeve note, through references to nuclear weapons, overpopulation and pollution, serves to mislead the casual reader; no mention of the first two problems is made in the body of the text and the only pollutions considered are those associated with the Indian caste system!

Philosophers then may find this volume of interest as a source of unorthodox views concerning their particular disciplinary problems; views developed and justified in reasonable accord with the current rules of the philosophical game. But the interest of the general reader will doubtless centre upon those papers which attempt to generate concrete value implications from natural science.

For Albert Szent-Gyorgyi, science is imbued with a characteristic spirit and morality. In contrast to the spirit of greed, lust and domination characteristic of political thought our society must be governed by the standards of human solidarity and mutual respect found within science; the enormous power provided by science can only be controlled and put to good ends in the light of the spirit of science itself. How many people today are able to share this vision of science, this faith in the scientific spirit? It enables Szent-Gyorgyi, innocent of all irony, to write: “To solve a problem meet it with a cool head, with uncompromising intellectual honesty, unbiased by greed, fear or hatred. . . . If you have an adversary, look upon him with respect as your associate with whom, together, you have to find the best solution. If this spirit would prevail at the peace talks in Paris, peace in Vietnam could rapidly be achieved.”

Thomas Colwell takes the results of science rather than its spirit as an objective basis for values and morality. Ecological limitations effectively circumscribe human values: “The ends which we propose must be such as to be compatible with the ecosystems of Nature.” Philosophers must create a new formulation of the relationship of man to nature in keeping with the findings of ecology. Such a synthesis, through re-education, could alter our “conceptual environment” and thereby the practical activity that constitutes our treatment of nature.

In their different ways, Colwell and Szent-Gyorgyi illustrate a view of moral problems which pervades this entire volume. Both believe that moral change can be produced by identifying or inventing the values which society “needs” and proliferating them as a system of concepts

and principles. Some authors, including Caldwell, are aware of obstacles or difficulties in such a process although these are never specified or discussed. But totally lacking is any awareness of the relationship between beliefs and social structure. What people believe or value depends on who they are, what social roles they occupy. Moral change is inextricably linked with changes in social structure, as a century of anthropological research has made clear. The ethical practices of societies are not invented by individuals, nor can they be changed by abstract argument. BARRY BARNES

## Behavioural Psychology

*Behavioural Worlds: The Study of Single Cases.* By P. G. Herbst. Pp. xiv + 248. (Tavistock: London and Sydney, November 1970.) £2.50.

THE empirical generalizations obtained from psychological research are often weak ones, for example finding correlations of 0.25 (accounting for 6 per cent of the variance). There are various possible reasons for this—the measurements may be unreliable, there may be many other factors operating and so on. A number of psychologists have begun to suspect that the reason is more fundamental in some way; Herbst maintains in this book that psychological laws are of the type  $X = aY$ , where the parameter  $a$  is different for each person, or  $X = f(Y)$ , where the function  $f$  varies between persons. If this is the case then the usual kind of research using groups of subjects is inappropriate, and studies must be made of individuals.

Two such studies are reported in this book, using statistical procedures applicable to individuals. The first is a study of an autonomous work group (reported in a previous book): repeated measures were made at different points in time of output, satisfaction, and other response measures; correlations were then found across time for each pair of measures, and partial correlations found with other variables held constant. Theoretical postulates were developed which were consistent with the patterns of results obtained. This



is presented, however, as a general theory of socio-technical systems, on the basis of a small amount of illustrative data from one working group. The second study is of the performance of a class of school children. Here the analysis is "cross-sectional" rather than "longitudinal": correlations are obtained for individuals across different school subjects, between variables such as work effort, boredom and anxiety. It was found that these correlations were quite different for different individuals in the form. In neither of these investigations are details given of the number of observations on which the statistics were based, the levels of statistical significance, or the way the measurements were made. In each case all the variables were response variables, and no attempt was made to link them with casual variables—such as individual profiles of ability in different subjects, the ability and style of teaching of different teachers and so forth.

The basic problem faced by this book is an important one, and the two methods suggested are of some interest though one is not new, and the data presented are quite inadequate. No reference is made to the work of others who are thinking about the same problem.

Now there has been much debate over whether consistent personality traits can act as parameters; IQ acts in this way, for example, where speed of learning under different conditions is being studied; Eysenck thinks that extraversion, neuroticism and psychoticism can be used in the same way. On the other hand, apart from intelligence there is considerable inconsistency of individuals across different situations, and this is particularly true of social behaviour.

Behaviour is found to vary, furthermore, with the situation as well as with the person, and a number of investigators have been trying to apportion the variance observed between person, situation, and person-situation interaction. The author has found that persons are consistent for groupings of situations, which are unique for them.

Lastly, the idea of studying individuals experimentally or statistically is not new, though not much research has been done in this direction. The longitudinal covariation with time method was used in 1946 by Baldwin and Cattell (who are not mentioned). Shapiro has carried out series of experiments on single individuals. The aim of these studies, however, has been to discover the mechanisms operative in particular individuals, not to discover empirical laws.

The book contains several further miscellaneous chapters, which appear to be unrelated to the principal theme. There is a great deal of mathematics, none of which seems particularly helpful. And it is written in a highly obscure, private terminology, based on the concepts of Kurt Lewin.

MICHAEL ARGYLE

## Questions and Answers

*Explanation in the Behavioural Sciences.* Edited by Robert Borger and Frank Cioffi. Pp. xii+520. (Cambridge University: London, November 1970.) £5.00; \$15.

THIS book is a collection of essays about problems of explanation and interpretation in psychology and sociology; each essay is followed by a comment and an answer to the comment. This alternation of authors forms the "confrontations" referred to in the book's sub-title. Many of the authors and commentators are people of distinction within their field.

As is pointed out in the editor's preface, the French Academy of Sciences decided in 1775 that it would no longer examine contributions from authors purporting to square the circle; after reading this book I am inclined to say that I will no longer consider contributions containing chapters called "Is the brain a physical system?" It is an unfortunate characteristic of the "soft" sciences, which include much of psychology and sociology, that long perorations about their histories and frames of reference are published. In the "harder" sciences, such as physics, there is greater concern with the actual gathering and interpretation of real data from well-defined systems. It is my prejudice that many of the doubts expressed by some of the authors in this book are provoked by one of two causes: a lingering vitalism and a premature desire to describe complex systems whose attributes are not precisely defined and about which too little is known. The workings either of the human brain or of human society are of awe-inspiring complexities. A natural result is a feeling, not necessarily explicitly acknowledged, that particular aspects of behaviour are not accessible to experiment or ultimate understanding. All our experience in other fields of science militates against this hypothesis, so it is a little surprising to see its resurgence under several guises in this book. For this reason I find little of pedagogic or heuristic value in the book and am forced to consider it solely on its merits as an entertainment. It fails.

Many of the confrontations reduce to familiar arguments between "Little-endians" and "Big-endians": in view of the polemics involved I can only be thankful that, in this book, neither side is armed. One of the more pervasive Big-endian theses is that some phenomenon is more than the sum of its parts; the triumphant Little-endian response is that parts which have joined to form a system no longer exist as entities. Neither group is apparently aware of the possibility of non-linear interactions, which are particularly likely in biological systems, although

both groups use, rather uneasily, some of the terminology of modern mathematics. A typical situation to which these arguments are applied is that of "the depressed group": are its members necessarily individually depressed or not? The involved and opaque writing surrounding the ill-defined problems considered continually reminds me of *Private Eye's* caveat: "Or not, as the case may be." Here are two examples: "Marginal utility theory endows economic man with a refined discrimination apropos border-line cases" (p. 208); "My explanation (of a tendency to become an habitual criminal) is that these children have inherited a reticular formation which has high thresholds to incoming stimulation; hence it provides the cortex with too little arousal, and causes it to be rather slow and weak in mediating conditional responses which I hold to be fundamental in elaborating a 'conscience'" (p. 423).

I find the lack of new ideas or of intellectual precision disturbing and am forced to return to the preface where the editors helpfully mention Wittgenstein's comment that in psychology there are experimental methods and conceptual confusion and that problems and methods pass one another by. Both contentions are remarkably well illustrated by this book. There are, of course, exceptions: a few of the articles are reasonably clear and sensible, for example, those by Sutherland, Chomsky and Cioffi, but they do not make a book worth £5.00.

ANTHONY ROBERTSON

## Machine Intelligence

*Progress of Cybernetics.* Vol. 1: Main Papers, The Meaning of Cybernetics, Neuro- and Biocybernetics. Edited by J. Rose. (Proceedings of the First International Congress of Cybernetics, London, 1969.) Pp. xiv+521. (Gordon and Breach: London and New York, September 1970.) \$24.50; £10.00.

THESE are the proceedings of a meeting held in London in 1969, which the editor calls the First International Congress of Cybernetics. The editor admits that some "somewhat fatuous contributions were included in order to bring to the surface certain undesirable accretions". He does not elaborate. "A mature science has to be able to live and cope with those who are trying to jump on the bandwagon and use it as a vehicle for their exuberant claims," states the editor. Now this may well be, but is it the best way to cope with this problem by adding to the ever-growing load of papers which the serious worker must scan?

Boulanger asks how can we discuss intelligent machines without defining

intelligence. I can find no definition in his paper. He suggests that information transmission needs energy, the human cannot create energy, so the human cannot create information! Pask states, "a human being does not so much respond to stimuli as interpret certain states of his environment as posing problems which he makes an attempt to solve". Is matter not atomic because this book moves entire, then?

Walter contrasts the hardware and software of the machine with the "wetware" of the brain. Ashby considers the coordination of a task organized in sub-groups. Beer suggests improved methods of organization of state and industry. As George states, in industry, "relevancy is the essence". Mays raises the old "can machines think?" problem. Foster seems to suggest that cybernetics demonstrates the existence of life after death. Malic elaborates on the theme of entropy consumed by life. Klir discusses general systems theory, but agrees with Boulding that "all we can say about practically everything is almost nothing".

Muses gives some interesting mathematics. But is this really cybernetics? Helvey has produced an interesting educational syllabus for cybernetics. Using plenty of italics, Challier describes cybernetics as the "science of sciences and the safeguard of man"! The printed paper by Sister Stella Mary makes it all the more tragic that, apparently, she was not allowed to attend the conference. Jones presents a useful collection of examples of positive feedback in nature. Like most of the workers in his particular section of the field of cybernetics, Kvitanishli gives us no practical details of his perceptron networks. Kogan concludes, very truly, that there are more questions than answers when considering brain organization.

Perhaps the above very short selection gives some idea of the variety of the papers presented at the conference. The papers mentioned above have all been from the Main Papers and from the section on "The Meaning of Cybernetics". The number of papers printed is 105, and they even include two which take the form of discussion and criticism of works of art. Clearly, Wiener's unfortunate definition of cybernetics can be twisted to cover almost any topic.

Those of us who attended the conference have only now been able to read the complete set of papers. Looking back, I feel that it is a great pity that it was not possible to prepare preprints of the papers. With so many simultaneous sessions, it was difficult, if not impossible, to select meetings for attendance.

The organizers certainly did a good job, but are not large international

meetings such as this better organized by the existing national learned societies, who have secretariats especially for such functions? It is possible also that fewer irrelevant papers might then be accepted and published.

JOHN F. YOUNG

## An Aspect of Virology

*Medical Virology*. By Frank Fenner and David O. White. Pp. xviii + 390. (Academic: New York and London, October 1970.) £4.45.

A NOTABLE lack felt by professional virologists and virology students alike is a succinct, reliable and comprehensive textbook. Excellent reference books, monographs and symposia are available to cover a wide range of different fields within the general area of virology, but a really good textbook of manageable length is hard to find.

It is a pleasure, therefore, to welcome Fenner and White's *Medical Virology* which provides not only such a textbook but also extensive reference coverage within the large field designated by its title and in related neighbouring fields as well. This is achieved by devoting well over half the book to an admirable survey of the principles and techniques of virology in general, and keeping the smaller second part of the book for a consideration of individual families of human viruses. Each chapter in Part I provides a masterly review of a topic vital to an understanding of virology. The style is clear and simple, extremely complex problems are made readily comprehensible and in areas of doubt a synthesis of the controversial points of view seems always somehow to be followed by a firm word of guidance from the authors. It is hard to envisage a better balanced or more useful introductory textbook for a complicated subject. To make the chapters even more valuable, each is followed by a recapitulation summary and a list giving guidance for further reading.

Despite the risk of writing a rave notice, I am still filled with wonder on reflecting on the seeming ease with which extremely complicated topics like viral genetics, virus multiplication or the effects of viruses on cells have, in the space of 10–15 pages, been reduced to an orderly survey of the basic facts involved, together with suitable explanations of their significance. It is also a pleasure to note that the diagrams and illustrations, whether photomicrographs or electron micrographs, are of an extremely high quality, well chosen and highly relevant to the points they seek to illustrate. This book can be used, therefore, both as an introductory textbook and as a very reliable and solid

reference book with which to start out on a quest for information on some specialized field in animal virology.

With regard to Part II, systematized surveys or catalogues, whether it be in plant nomenclature, bacterial classification, or the listing and describing of viruses, tend, by their very nature, to be boring. Once again, Fenner and White's new book scores a minor triumph; this section, too, by virtue of the engaging and direct style of the authors, remains readable and extremely interesting throughout. All in all, this book is to be highly recommended to students and established workers alike. It will for long be a "must" on the shelves of all those connected, however remotely, with virological topics, and it should be stressed that despite its title it is not in the least limited to material likely to be of interest only to medical workers. The basic scientist, the research diagnostician, the physician and the epidemiologist will, together with many others, find that an outstanding textbook in this field has now been provided.

M. A. EPSTEIN

## Red Cells and White

*Regulation of Hematopoiesis*. Edited by Albert S. Gordon. Vol. 1: Red Cell Production. Pp. xviii + 1-766 + 9. Vol. 2: White Cell and Platelet Production. Pp. xviii + 767-1658. (Appleton-Century-Crofts: New York, August 1970.) Two volumes: \$78.50.

THE scope of these books reflects the development of haematology from the study of clinical material, entirely for medical purposes, to an important branch of experimental biology. Studies of haematopoiesis at the tissue, cellular, and molecular levels continue to make fundamental contributions to developmental and molecular biology, in particular those areas concerned with cellular regulatory systems, and may provide insights of increasing value to clinicians in preventing or controlling a variety of haematopoietic disorders.

In the first volume, comparative haematology is introduced by a detailed study of insect haemocytopoiesis and a brief commentary on haematopoietic mechanisms in non-mammalian vertebrates. Haematopoietic regulation is placed in the context of an organized tissue by articles on bone marrow and spleen histophysiology. Various aspects of the haematopoietic stem cell, control and kinetics of its differentiation, and tissue interactions *in vivo* and *in vitro*, are usefully reviewed in five chapters. Ultrastructural and kinetic aspects of erythropoiesis are supplemented by chapters on kinetic models and general application of control theory to red cell differentiation. Preparation and chemistry of erythro-



poietin, its bioassay and standardization, precede general chapters on erythropoietin physiology and mode of action, and there is a useful section on non-erythropoietin dependent erythropoiesis. Foetal and neonatal erythropoiesis is examined in terms of differences in control between prenatal and adult animals, and the occurrence and significance of foetal haemoglobins. Prenatal erythropoiesis also receives considerable attention in a valuable chapter on genetic abnormalities of erythropoiesis in mice. Discussion of clinical derangements of human erythropoiesis due to mutant genes receives minimal attention, however, although there is a chapter on polycythaemia. Biochemical aspects of erythropoiesis are summarized in chapters on regulation of iron metabolism, role of transferrin, and haem and globin synthesis.

The second volume is devoted entirely to white cell and platelet production. The ultrastructure of developing, normal and diseased granulocytes is discussed in two introductory chapters, and there is also a discussion of kinetic analysis of normal granulocyte production and release. Morphological aspects and kinetics of neutrophil and eosinophil production and mobilization receive detailed attention in four chapters. Sections on metabolism of resting and phagocytosing leucocytes provide the physiological background for discussions of the role of leucocytes in inflammation and immunology, including their passage through blood vessel walls, and subsequent migration. Various aspects of lymphocyte production are dealt with in detail, beginning with the ultrastructure of lymphocytes and their development. Discussions of the regulation and kinetics of lymphocyte differentiation *in vivo* and *in vitro* complement structural and functional descriptions of the lymph node and lympho-myeloid complex and their role in the immune response. Abnormalities of white cell proliferation are discussed in chapters dealing with the value of experimental leukaemias, kinetics and biochemistry of leukaemic leucocytes and lymphocytes, and the immuno-proliferative disorders. Ultrastructural and physiological aspects of platelet production in three chapters complete this volume.

In general the editor has succeeded in integrating some thirty articles in each volume into a coherent treatise. Both are illustrated to a high standard, including many photomicrographs. Inevitably, in a work of this scope it is possible to fault the relative attention given to different topics. Perhaps, most seriously, in Volume I recent advances in understanding the regulation of haemoglobin synthesis at the molecular level receive little attention, and, in Volume II, there is little discussion of the role viruses may play in abnormal cell proliferation. Extensive reference lists follow each chapter, and are a valuable feature of both volumes,

but there is no overall author index, and the subject indices are disappointingly inadequate.

These books will be of great value to experimental haematologists in relating their data to a coherent view of haematopoietic regulation, and the editor's hope that his labours will stimulate further research is likely to be fulfilled.

R. J. COLE

## Lymph and Lymphocytes

*Lymphatics, Lymph and the Lympho-myeloid Complex.* By Joseph Mendel Yoffrey and Frederick Colin Courtice. Pp. xviii + 942. (Academic: London and New York, November 1970.) £12.50; \$35.

THIS is a new book which has succeeded two previous editions on the same subject with a similar name, *Lymphatics, Lymph and Lymphoid Tissue*. The principal advances in this subject, however, have necessitated a new text with rather different emphasis. The book is very large and comprehensive and is an attempt to unify as a system the lymphocytes and their source of origin and their lymphatic vessels. The result is a very long book ranging through many different subjects. The embryology of lymphatics is discussed together with the physiology of lymph flow and the composition of lymph. Comparative aspects in vertebrates are considered together with lymph vessels for each individual organ. There are sections on radiology, and pathology of lymphatics; particularly the results of infection and tumour.

The lymphocyte is discussed from all points of view, namely, morphology, movement, enzyme production, response to mitogens and the relationship of the lymphocyte to the macrophage. The lymph node is similarly treated from a morphological and functional point of view and the immunological changes that occur in relation to antigenic stimulation and homotransplantation are illustrated. There are chapters on lymphocyte production and lymphocyte migration and the relationship of the thymus to the rest of the lymphatic system. There is a final section on laboratory technique in determination of cell viability, lymphocyte culture, production of lymphocyte suspensions and cell transfusion, and the shielding of lymphocytes from damage of irradiation.

The book is an excellent work of reference. The bibliography, if not comprehensive, is certainly adequate and it is easy to find references, for they are recorded both in chapters and at the end of the book in an author index. The work represents immense effort of scholarship and will be an essential volume in all laboratories undertaking biological research. My only criticism would be that with such a wide scope, individual subjects cannot possibly

be dealt with in great detail, but this is not presumably the objective of the text which is more in the form of a review and, as such, can be confidently recommended.

R. Y. CALNE

## Continents Adrift

*Continental Drift: A Study of the Earth's Moving Surface.* By D. H. Tarling and M. P. Tarling. Pp. 112 + 4 plates. (Bell: London, February 1971.) £1.50.

ANYONE who chooses to write a popular account of continental drift, sea floor spreading, plate tectonics and similar topics variously lumped together these days as the "new global tectonics" must face up to a problem. There is already one book covering this ground at a similar level<sup>1</sup>—and a book, moreover, to which so many superlatives have been applied in review that the adman's view of the latest Hollywood epic looks pale by comparison. None of this would matter too much, of course, were the praises sung to Takeuchi *et al.* misplaced. But they were not. The result is that the Tarlings are attempting to surpass a feat which is wellnigh insurpassable and which will almost certainly not be surpassed for many a year to come.

That the Tarlings have not quite succeeded in this should not worry them too much, however. The comparison is reasonable and even inevitable; but it is also possible to judge a book on its own merits. To say that Brahms did not quite surpass Beethoven is not to suggest that the former is not worth an audience. In fact, the Tarlings have produced a rather good survey of our present view of the Earth as a dynamic body. Beginning with the prophets of continental drift they take us through the evidence from geometry (the "continental jigsaw"), the Earth's crust (the "picture on the jigsaw"), ancient environments and climates, and palaeomagnetism to sea floor spreading and plate tectonics. The final three chapters then deal with the timing, causes and significance of the drift phenomenon.

If I have any misgivings at all, it is just that the Tarlings have a slight tendency to oversimplify and perhaps even overdramatize. The idea that Francis Bacon first spotted the congruency between the west coast of Africa and the east coast of South America makes a fascinating little story but owes more to myth than reality. More seriously perhaps, surely it is misleadingly optimistic to suggest that "in the very near future it should be possible to control many earthquakes"? The Joadian response to the very near future hardly covers that one. But these are small points. The book as a whole rates five stars.

PETER J. SMITH

<sup>1</sup> Takeuchi, H., Uyeda, S., and Kanamori, H., *Debate About the Earth* (Freeman, Cooper, San Francisco, 1967, revised edition 1970.)

<sup>3</sup> Teräväinen, H., *Experientia*, **25**, 524 (1969).



# Announcements

## University News

**Dr M. J. O'Hara** has been appointed to a personal chair of petrology in the **University of Edinburgh**.

**Dr R. J. Knops**, University of Newcastle upon Tyne, has been appointed to the chair of mathematics at **Heriot-Watt University**, in succession to **Professor R. Smart**, who retires this year.

The following appointments have been made in the **University of London**: **Dr R. P. Dales** has been appointed to the chair of zoology tenable at Bedford College; **Dr P. J. Lachmann** has been appointed to the chair of immunology tenable at the Royal Postgraduate Medical School and **Dr Walter Plowright** has been appointed to the chair of veterinary microbiology and parasitology tenable at the Royal Veterinary College. The following titles have been conferred: professor of embryology, on **Professor F. Beck**; professor of dental anatomy, on **Professor R. W. Fearnhead**; and professor of physiology, on **Professor W. R. Keatinge**, in respect of their posts at the London Hospital Medical College; professor of computer systems, on **Dr P. T. Kirstein**, in respect of his post at the Institute of Computer Science; professor of epidemiology and preventive medicine, on **Professor G. A. Rose**, in respect of his post at St Mary's Hospital Medical School; professor of computer science, on **Professor P. A. Samet**, in respect of his post at University College.

**Dr K. W. Brittan** has been appointed to the newly created Rank chair of instrument science and technology at **Loughborough University of Technology**.

## Miscellaneous

The research medal of the **Royal Society of Victoria** has been awarded to **Professor G. Burnstock**, University of Melbourne, for his work on the autonomic innervation of smooth muscle.

**Academician I. M. Vinogradov**, mathematician and member of the **Academy of Sciences of the USSR**, has been awarded the Lomonosov gold medal of the Academy.

The **Edinburgh Geological Society** has awarded the Clough medal to **Professor F. H. Stewart**, University of Edinburgh, and the Clough award to **Dr P. Toghil**, British Museum (Natural History). Applications for a grant from the Clough memorial fund for geological research in Scotland and the North of England should be sent to the secretary of the Society, 19 Grange Terrace, Edinburgh EH9 2LF.

The **Linnean gold medal** has been awarded to **Dr J. E. Smith**, director of the Marine

Biological Association, Plymouth, for his work in zoology and to **Dr C. R. Melcalfe**, formerly keeper of the Jodrell Laboratory, Royal Botanic Gardens, for his work in botany. The H. H. Bloomer award to an amateur naturalist has been made to **Dr John G. Dony**, author of *Floras of Bedfordshire and Hertfordshire*.

**Harkness fellowships** of the Commonwealth Fund for 1971 have been awarded to the following scientists: **C. J. Adams**, University of Oxford (chemistry); **D. J. Broadhurst**, University of Sussex (physics); **D. J. Collop**, University of Cambridge (applied mathematics); **J. H. Danskin**, University of Edinburgh (physics); **J. S. Halliday**, University of Cambridge (electronics); **E. F. Hawkins**, University of Sheffield (zoology); **S. A. McKilligan**, University of Aberdeen (mathematics); **N. C. Price**, University of Oxford (biochemistry); **T. E. H. Walker**, University of Oxford (chemistry); **C. G. Wynn-Williams**, University of Cambridge (astronomy).

Awards for 1971 have been announced by the **Council of the Institute of Physics and the Physical Society**: Guthrie medal and prize, to **Mr J. A. Ratcliffe**, formerly of the Radio Research Station; Glazebrook medal and prize, to **Dr F. E. Jones**, Mullard Ltd; Thomas Young medal and prize, to **Professor C. G. Wynne**, Imperial College of Science and Technology; Maxwell medal and prize, to **Dr J. B. Taylor**, Culham Laboratory, UKAEA; Charles Chree medal and prize, to **Mr D. G. King-Hele**, Royal Aircraft Establishment; Duddell medal and prize, jointly to **Dr V. E. Cosslett** and **Dr K. C. A. Smith**, University of Cambridge; Charles Vernon Boys prize, to **Dr M. Hart**, University of Bristol; Bragg medal and prize, to **Mr G. R. Noakes**, formerly of Uppingham School; A. B. Wood medal and prize, to **Dr R. E. Apfel**, Harvard University.

The following were elected **Fellows of the Royal Society** on March 18: **Professor N. H. Ashton**, Institute of Ophthalmology, University of London; **Dr J. H. Beynon**, Dyestuffs Division of Imperial Chemical Industries Limited; **Mr B. B. Boycott**, MRC; **Professor A. Carrington**, University of Southampton; **Professor D. H. Copp**, University of British Columbia; **Dr G. S. Dawes**, Nuffield Institute for Medical Research, Oxford; **Professor M. E. Fisher**, Cornell University; **Professor D. V. Glass**, London School of Economics, University of London; **Dr J. B. Gurdon**, University of Oxford; **Dr B. S. Hartley**, MRC; **Mr Lionel Haworth**, Rolls Royce Limited; **Professor R. Hide**, Meteorological Office; **Professor J. F. C. Kingman**, University of Oxford; **Professor J. Mandelstam**, University of Oxford; **Dr E. H. Mansfield**, Royal Aircraft Establishment; **Dr W. Marshall**, UKAEA; **Professor J. L. Monteith**, University of Nottingham;

**Professor F. R. N. Nabarro**, University of the Witwatersrand; **Dr A. C. Neish**, National Research Council of Canada; **Professor P. R. Owen**, Imperial College, University of London; **Professor J. C. Polanyi**, University of Toronto; **Dr A. F. Posnette**, East Malling Research Station; **Dr R. E. Reason**, Rank Precision Instruments Limited; **Dr F. G. Rees**, University College of Wales; **Mr J. D. Rose**, Imperial Chemical Industries Limited; **Professor G. W. Series**, University of Reading; **Professor R. M. Shackleton**, University of Leeds; **Professor T. I. Shaw**, Queen Mary College, University of London; **Professor B. C. L. Weedon**, Queen Mary College, University of London; **Dr A. M. Wetherell**, CERN; **Professor H. B. Whittington**, University of Cambridge; **Professor D. R. Wilkie**, University College, University of London.

## Errata

IN the article "Fission Track Dating of Archaeological Materials from Japan" on page 242 of this issue of *Nature*, the first sentence on page 243 should read "Zircon required from 1 to 2 min in concentrated  $\text{H}_3\text{PO}_4$  at 450–500° C . . .".

IN the article "Search for a Human Breast Cancer Virus" by Dan H. Moore *et al.* (*Nature*, 229, 611; 1971), the word "man" on line 3 of the first paragraph should read "mice". The penultimate line of the fourth paragraph should read "Gentamycin (Schering 0.01 ml./ml. of milk) . . .".

IN the article "Crystal Structure of  $\text{Cu}(\text{adenine})_2\text{Cl}_2 \cdot 3\text{H}_2\text{O}$  and Bridging Capability of Adenine" by P. de Meester, D. M. L. Goodgame, K. Angela Price and A. C. Skapski (*Nature*, 229, 191; 1971), the abbreviation EPR was mistakenly translated as electron proton resonance. This should, of course, have read electron paramagnetic resonance.

IN the article "Parameter Sensitivity as a Criterion for Evaluating and Comparing the Performance of Biochemical Systems" by Michael A. Savageau (*Nature*, 229, 542; 1971), the paragraph immediately following Fig. 1 should read as follows: "The following definition of parameter sensitivity meets these requirements and avoids the difficulties encountered in measuring the effectiveness of a biochemical control system that I have mentioned. (In this definition a parameter will refer to a property of the system that is normally a fixed quantity, and may be considered characteristic of the system. In different systems, or in different environments, parameters may assume different but fixed values. For example, in a simple enzyme-catalysed reaction the Michaelis constant  $K_m$  would be a parameter of the system, whereas the concentration of the substrate would be

considered a variable.) In the steady state the relative variation (or percentage change) in some property  $X$ , related to the system function in question, with respect to the relative variation of a parameter  $\alpha$ , characteristic of the system, is defined as the parameter sensitivity of  $X$  with respect to  $\alpha$ , that is . . . .”.

## International Meetings

March 29–April 2, **New Problems, New Techniques and New Concepts in Statistical Mechanics**, Chicago (Professor S. A. Rice, James Franck Institute, University of Chicago, Chicago, Illinois 60637, USA).

March 31–April 2, **Joint Meeting of the Genetical Society and the Virus Group of the Society for Genetical Microbiology**, Cambridge (Dr Michael Ashburner, 0223 56894).

May 3–7, **Automatic Indexing and Information Retrieval**, London (Mr C. W. Cleverdon, Cranfield Institute of Technology, Cranfield, Bedford, England).

May 4, **Crop Protection**, Ghent (Faculteit van de Landbouwwetenschappen, Coupure Links 533, B-9000 Ghent, Belgium).

May 6–7, **Computer Culture**, University of Bradford (University of Bradford, Bradford, Yorkshire BD7 1DP).

May 18–21, **International London Electronic Component Show**, London (Siemens (United Kingdom) Ltd, Publicity Dept., Great West House, Great West Road, Brentford, Middlesex).

June 4, **Two Phase Morphology of Block Co-polymers**, University of Essex (Prof. M. Gordon, Dept. of Chemistry, University of Essex, Wivenhoe Park, Colchester, Essex).

June 6–10, **Tissue Culture Association Annual Meeting**, Lake Placid, New York (Dr Vincent J. Cristofalo, The Wistar Institute, 36th Street at Spruce, Philadelphia, Pennsylvania 19104, USA).

June 13–15, **Canadian Society of Plant Physiologists Annual Meeting**, Toronto (Professor Dorothy Forward, Department of Botany, University of Toronto, Toronto 181, Ontario, Canada).

June 13–16, **Cranfield Fluidics Conference**, University of Uppsala (H. S. Stephens, Secretary, 5CFC Organizing Committee, British Hydromechanics Research Association, Cranfield, Bedford, England).

June 14–18, **Theoretical Biology and Biomathematics**, Proctor Academy, Andover, New Hampshire (Dr A. M. Cruickshank, Director, Gordon Research Conferences, Colby Jnr. College, New London, New Hampshire. Tel. 603 526 2870).

June 14–18, **Radiology in Amsterdam**, International Congress Centre, Amsterdam (Congress Secretariat, c/o Holland Organizing Centre, 16 Lange Voorhout, The Hague).

June 17–27, **Telecom**, Geneva (International Telecommunications Union, Place des Nations, Geneva, Switzerland).

June 21–26, **Electromagnetic Interactions**, Trieste (A. M. Hamende, International Centre for Theoretical Physics, Miramare, PO Box 586, 34100 Trieste, Italy).

June–July, **World Administrative Radio Conference for Space Telecommunications**, Geneva (International Telecommunications Union, Place des Nations, Geneva, Switzerland. Tel. 34 70 00).

June 21–July 30, **Advanced Study in Gerontology**, S. California (Summer Institute Coordinator, Gerontology Center, University of Southern California, University Park, Los Angeles, California 90007).

June 21–July 30, **Community Mental Health and Ageing**, Southern California (Summer Institute Coordinator, Gerontology Center, University of Southern California, University Park, Los Angeles, California 90007).

July 6–9, **Major Loss Prevention in the Process Industries**, Newcastle upon Tyne (Institution of Chemical Engineers, 16 Belgrave Square, London SW1).

July 6–17, **Introduction to Techniques in Electron Microscopy**, Middletown, NY (G. Mokotoff, Orange County Community College, Middletown, NY 10940, USA).

July 7–9, **The Structure of Matter**, Physics Dept., University of Canterbury, Christchurch (Prof. B. G. Wybourne, Physics Dept., University of Canterbury, Christchurch, New Zealand).

July 12–19, **Relativity and Gravity**, Trieste (A. M. Hamende, International Centre for Theoretical Physics, Miramare, PO Box 586, 34100 Trieste, Italy).

July 19–23, **Molecular Energy Transfer**, Cambridge (University of Cambridge, Dept. of Physical Chemistry, Lensfield Road, Cambridge CB2 1EF).

July 25–30, **The Engineering Properties of Sea Floor Soils and their Geophysical Identification**, Seattle (M. A. Sherif, Department of Civil Engineering, University of Washington, Seattle, Washington 98105, USA).

July 25–30, **Pure and Applied Chemistry**, Boston (American Chemical Society, 1155 Sixteenth Street, NW Washington DC 20036, USA).

July 26–30, **Physics of Electronic and Atomic Collisions**, Amsterdam (Organisatie Bureau Amsterdam NV Postbus 7205, Amsterdam, Netherlands).

July 27–August 8, **Molecular and Developmental Biology**, Sicily (H. G. Zachau, 8 Munchen 15, Goethestrasse 33, West Germany).

August 2–4, **Operations Research**, Chicago (The Graduate Library School, University of Chicago, 1100 East 57th Street, Chicago, Ill. 60637).

## British Diary

### Monday, March 29

**Land Pollution** (6 p.m.) Sir Frank Fraser Darling, Royal Society of Arts, at John Adam Street, Adelphi, London WC2. (Third of four Cantor Lectures on “Problems of Pollution”).

### Tuesday, March 30

**Enzymes in Industry** (symposium) Birmingham University Chemical Engineering Society, Postgraduate Section, at the University.

**New Materials in Architecture** (discussion meeting) Institution of Metallurgists, at the Department of Metallurgy, Imperial College, London SW7.

**The Influence of the Industrial Training Boards on the Electricity Industry** (5.30 p.m. discussion) Institution of Electrical Engineers, at Savoy Place, London WC2.

**The M21/40 Computer for Pembroke Power Station** (5.30 p.m.) Mr R. I. Scott-Kerr, Institution of Electrical Engineers, at Savoy Place, London WC2.

### Wednesday, March 31

**165th Meeting** (three days) Genetical Society, jointly with the Virus Group of the Society for General Microbiology, at the University of Cambridge.

**Environmental Engineering Aspects of Pollution Control** (symposium, two days) Society of Environmental Engineers, at Earls Court, London.

**Hormonal Control of Gene Action in Cultured Cells** (4.30 p.m.) Dr Gordon Tomkins (University of California), Medical Research Council, at the National Institute for Medical Research, Mill Hill, London NW7.

**Meat in the Future—Problems and Solutions** (two-day symposium) Institute of Biology; the Institute of Food Science and Technology; the Institute of Refrigeration and the Institute of Meat (in London).

**Plasma Torches** (colloquium) Institution of Electrical Engineers, at the University, Sheffield.

**The Impact of Colour in Commerce** (third Scottish symposium, three days) Colour Group (Great Britain), at the University of Edinburgh.

### Thursday, April 1

**Electrical Heating in Buildings** (two-day conference) Institution of Electrical Engineers, in association with the Institution of Heating and Ventilating Engineers, at the Institution of Electrical Engineers, Savoy Place, London WC2.



**Future Techniques for Cockpit Display** (6 p.m.) Mr D. R. Evans and Captain D. S. Kirkland, Institution of Electronic and Radio Engineers, at 9 Bedford Square, London WC1.

**Perfumes** (7 p.m.) Mrs Lilian Verney, Oil and Colour Chemists' Association, at the British Rail School of Transport, London Road, Derby.

## Friday, April 2

**Analytical Uses of Solid Electrolytes** (symposium) Society for Analytical Chemistry, in the Department of Mechanical Engineering, Imperial College, London SW7.

## Monday, April 5

**Elementary Particle Physics** (three-day conference) Institute of Physics and the Physical Society, at the University of Lancaster.

**Pollution in Context** (6 p.m.) Dr Martin W. Holdgate, Royal Society of Arts, at John Adam Street, Adelphi, London WC2. (Last of four Cantor Lectures on "Problems of Pollution".)

**Testing Methods and Techniques** (5.30 p.m. discussion) Institution of Electrical Engineers, at Savoy Place, London WC2.

**Third National Atomic and Molecular Physics Conference** (four days) Institute of Physics and the Physical Society, at the University of York.

## Reports and Publications

not included in the monthly Books Supplement

### Great Britain and Ireland

Inside Colleges of Further Education. By Adrian Bristow. Pp. vi+149+26 photographs. (Department of Education and Science.) (London: HMSO, 1970.) 30p. [181]

Natural Environment Research Council. Merlewood Research Station—Report for 1967–1969. Pp. 66+10 plates. (Grange-over-Sands: The Nature Conservancy, 1970.) 50p. [181]

Ministry of Agriculture, Fisheries and Food. Technical Bulletin No. 22: Internal Stages of Bulb Development. Pp. iv+12. (London: HMSO, 1970.) 17½p. [191]

The Wildlife of Northumbria. By William Balmain. (Northern Wildlife Book No. 1.) Pp. 32. (Newcastle upon Tyne: Frank Graham, 6 Queen's Terrace, 1971.) 30p. [191]

Proceedings of the Royal Irish Academy. Vol. 70. Section A. No. 5: A Note on L-Strips. By B. G. Eke. Pp. 33–34. 7½p. No. 6: Decay Processed in a High Density Argon Afterglow. By B. V. Donovan and M. C. Sexton. Pp. 35–46. 17½p. No. 7: On the Saturation of Non-Factored Current Algebra at Infinite Momentum with Simplified Angular Condition. By I. Khan, U. H. Niederer and L. O'Riadaigh. Pp. 47–58. 20p. No. 8: The Frequency Distribution of Atmospheric Condensation Nucleus Concentrations. By E. I. Hayes. Pp. 59–70. 17½p. Vol. 70, Section. No. 5: The Geology of the Toombeola District, Connemara, Co. Galway. By B. W. Evans and B. E. Leake. Pp. 105–140+ plates 4–7. 77½p. No. 6: The Quaternary Deposits Between Fenit and Spa on the North Shore of Tralee Bay, Co. Kerry. By G. F. Mitchell. Pp. 141–162+ plate 8. 35p. No. 7: The Lichens and Lichen Parasites of Derryclare Wood, Connemara. By Ann C. M. Folan and M. E. Mitchell. Pp. 163–170. 10p. No. 8: Movements of Salmon Around Ireland—X. from Carnlough and Torr Head (1969). By K. U. Vickers and G. D. Ferris. Pp. 171–178. 12½p. No. 9: Antitubercular Substances—XXII. Remino-Phenazines—The Effect of Further Substitution. By V. C. Barry, J. G. Belton, M. L. Conalty and Mary McInerney. Pp. 179–194. 25p. No. 10: The Lower Palaeozoic Rocks of the Louisburgh Area, Co. Mayo. By W. E. A. Phillips, R. B. Rickards and J. F. Dewey. Pp. 195–210+ plates 9 and 10. 27½p. (Dublin: Royal Irish Academy, 1970.) [191]

The Wellcome Foundation Limited. Annual Report and Accounts Year ended 31st August, 1970. Pp. 32. (London: The Wellcome Foundation, 1971.) [261]

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 268, No. 1192 (4th February, 1971): A Discussion on the Petrology of Igneous and Metamorphic Rocks from the Ocean Floor. Organized by Sir Edward Bullard, FRS, J. R. Cann and D. H. Matthews. Pp. 381–750+ plates 1–10. £10; \$26. Vol. 269, No. 1194 (5th February, 1971): Multicomponent Adsorption in Continuous Countercurrent Exchangers. By Hyun-Ku Rhee, R. Aris and N. R. Amundson. Pp. 187–215. £8.80; \$20.50. Vol. 269, No. 1195 (5th February, 1971): On the Motion of Space Charge in a Dielectric Medium. By J. H. Calderwood and B. K. P. Scaife. Pp. 217–232. £0.40; \$1.05. Vol. 269, No. 1196 (5th February, 1971): Electronic Aids to Night Vision. By P. Schagen. Pp. 233–263+ plates 4 and 5. £0.95; \$2.45. Vol. 269, No. 1197 (5th February, 1971): The Profiles of Axially Symmetric Menisci. By J. F. Padday. Pp. 265–293. £0.75; \$1.97. (London: The Royal Society, 1971.) [102]

### Other Countries

Bulletin of the Museum of Comparative Zoology, Harvard University. Vol. 140, No. 6: The Systematics and Zoogeography of the Unionidae (Mollusca: Bivalvia) of the Southern Atlantic Slope Region. By Richard I. Johnson. Pp. 263–450 (22 plates). Vol. 140, No. 7: Ecological-Behavioral Studies of the Wasps of Jackson Hole, Wyoming. By Howard F. Evans. Pp. 451–511 (5 plates). (Cambridge, Mass.: Museum of Comparative Zoology, Harvard University, 1970.) [111]

International Atomic Energy Agency, Vienna. Technical Reports Series, No. 121: Fertilizer Management Practices for Maize—Results of Experiments with Isotopes. (Results of a Four-Year Co-ordinated Research Programme of the Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture.) Pp. 78. (Vienna: IAEA; London: HMSO, 1970.) 78 schillings; £1.25; \$3. [111]

State of California. The Resources Agency, Department of Fish and Game. Fish Bulletins. No. 147: The Northern Anchovy (*Engraulis mordax*) and Its Fishery, 1965–1968. By James D. Messersmith. Pp. 102. No. 148: Effects of Artificial Destratification on Distribution of Bottom Organisms in El Capitan Reservoir. By Inland Fisheries Branch. Pp. 30. No. 149: The California Marine Fish Catch for

1968 and Historical Review 1916–68. By Richard F. G. Heimann and John G. Carlisle, Jr. Pp. 69. No. 150: A History of California's Fish Hatcheries 1870–1960. Pp. 92. (Sacramento, California: Office of Procurement, PO Box 20190, 1970.) [121]

Records of the Australian Museum. Vol. 28, No. 4: Two New Stomatopod Crustaceans from Australia. By Raymond B. Manning. Pp. 77–85. (Sydney: The Australian Museum, 1970.) [121]

New Zealand: Department of Health. Environmental Radioactivity in New Zealand—Oily Report April/June 1970 and Pacific Area Monitoring 27 July/9 October. Pp. 28. (Christchurch: National Radiation Laboratory, 1970.) [121]

National Academy of Sciences/National Academy of Engineering. Wastes Management Concepts for the Coastal Zone—Requirements for Research and Investigation. Pp. vii+126. (Washington, DC: National Academy of Sciences/National Academy of Engineering, 1970.) \$3.50. [121]

Companhia de Diamantes de Angola (DIAMANG). Serviços Culturais. Museu do Dundo. Publicações Culturais No. 80: Estudos de História (Ultra-marina e Continental). Présence du Portugal en Belgique (De Philippe d'Alsace à Léopold Ier). Par Eduardo Brazao. Pp. 196. (Lisboa: Companhia de Diamantes de Angola, 1970.) [121]

Geological Survey of Botswana. Geological Map—Quarter Degree Sheet 2227B: Selebi. (Lobatse: Geological Survey, 1970.) [131]

Annals of the South African Museum. Vol. 57, Part 3: Hyperidae (Crustacea: Amphipoda) Keys to South African Genera and Species, and a Distribution List. By R. I. Dick. Pp. 86. (Cape Town: South African Museum, 1970.) [131]

World Health Organization. Technical Report Series. No. 456: Training in National Health Planning—Report of a WHO Expert Committee. Pp. 59. 4 Sw. francs; 40p; \$1.25. No. 457: The Prevention of Perinatal Mortality and Morbidity—Report of a WHO Expert Committee. Pp. 60. 4 Sw. francs; 40p; \$1.25. (Geneva: WHO; London: HMSO, 1970.) [131]

US Department of the Interior: Geological Survey. Professional Paper 627-D: Urbanization and Its Effect on the Temperature of the Streams on Long Island, New York. By Edward J. Pluhowski. Pp. v+110. (Washington, DC: Government Printing Office, 1970.) \$1.25. [131]

Issues for Study in Cable Communications. By Arthur L. Singer, Jr. Pp. 19. (New York: Alfred P. Sloan Foundation, 1970.) [141]

US Department of the Interior: Geological Survey. Bulletin 1266: Bibliography of North American Geology, 1966. Pp. xi+1069. \$4.75. Bulletin 1262-E: Stratigraphy of the Morrison Formation and Structure of the Ambrosia Lake District, New Mexico. By Elmer S. Santos. Pp. iii+30+1 plate. Bulletin 1319-D: Mineral Resources of the Sawtooth Primitive Area, Idaho. By Thor H. Killsgaard, Val I. Freeman and Joseph S. Coffman. Pp. ix+174+2 plates. (Washington, DC: Government Printing Office, 1970.) [141]

Research and Development in State Government Agencies, Fiscal years 1967 and 1968. (National Science Foundation.) Pp. xi+93. (Washington, DC: Government Printing Office, 1970.) \$1. [141]

Politics and Society, Vol. 1, No. 1, November 1970. Pp. 1–150. Subscription rates: Institutional \$15; Individual \$10; Student \$6. (Los Altos, California: Geron-X, Inc., Publishers, 1970.) [151]

Medical Research: Priorities and Responsibilities. (Proceedings of a Round Table Conference organized by CIOMS with the assistance of WHO and UNESCO, Geneva, 8–10 October, 1969.) Pp. 154. (Geneva: WHO; London: HMSO, 1970.) 8 Sw. francs; 80p; \$2.75. [181]

US Department of Commerce. National Bureau of Standards Monograph No. 117: Hearing Aids. By Edith L. R. Corliss. Pp. iv+24. (Washington, DC: Government Printing Office, 1970.) \$0.35. [181]

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